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CONTENTS

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Larvicidal activity of Arecanut extracts (*Areca catechu*) on three medically important dipteran flies

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ABSTRACT: Aqueous and ethanol extracts of arecanut were screened for larvicidal activity by contact toxicity and diet incorporation methods against *Musca domestica*, *Chrysomya megacephala* and *Culex* sp. Ethanol extract showed maximum larvicidal activity against all the three insect species tested. The phytochemical analysis showed that ethnanol extract possessed many phytochemicals, which included carbohydrates, alkaloids, glycosides, phenolics and tannins. Mosquito larvae were more susceptible to the extracts than *C. megacephala* and *M. domestica* larvae. The LD_{50} of the ethanolic extract for *M. domestica*, *C. megacephala* and *Culex* sp. were 5.88, 1.86 and 0.072 mg /ml respectively.

KEYWORDS: larvicidal, arecanut, dipteran flies, mosquito

INTRODUCTION

The housefly *Musca domestica* L. and blow fly *Chrysomya megacephala* (F) are medically important insects worldwide. They are eusynanthropes and hence, closely associated with human environment for their entire development. Not only are the adult flies pestiferous, they also play a role as mechanical carriers of many pathogens eg. Viruses, bacteria and parasites to humans which lead to diseases such as typhoid, cholera, bacillary dysentery, amoebic dysentery, ophthalmia or diarrhoea (Wallace, 1971; Kasprzak and Majewska, 1981; Akinboade *et al.*, 1984; Agui, 2001; Graczyk *et al.*, 2001; Nayduch and Stutzenberger, 2001) the pathogens also stick on their bodies or get them disseminated via faeces. Their larvae can be the myiasis producing agents both in humans and animals. This results in economic loss particularly in the latter case and especially when the myiasis occurs in agronomic live stock (Kumarasinghe *et al.*, 2002). Mosquitoes are a large arthropod group which has a anopheline and culicine

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sub family, into which *Anopheles* and *Culex* belong. Encephalitis virus is transmitted by *Culex* sp.

For the past five decades fly control heavily relies on conventional chemical insecticides like organocholines, organophosphates and carbamates in both the public health care arena and agricultural areas. Although the chemical insecticides can effectively reduce adult fly populations, some side effects are created by the chemicals among which human health hazards, residues in food, and destruction of non-target organisms are very important (Ahmed *et al.*, 1981). The long persistence of such chemicals in the environment can result in their accumulation in animal tissues, which are magnified in food chains or food webs.

Botanical pesticides are possible alternatives in fly control (Bisseleua *et al.*, 2008). Some of the plant products viz., azadirachtin from *Azadirachta indica*, pyrethrin from *Chrysanthemum cineraiaefolium*, carvone from *Carum carvi* and allyl isothiocyanate from mustard and horseradish oil have received global attention due to their pesticidal properties and potential to protect several commodities (Hartmans *et al.*, 1995). Keeping these facts in mind, in the present investigation Arecanut extracts were tested for their antilarval activity with a view to protect stored grains from insect pests.

MATERIALS AND METHODS

About 100 g of tender arecanuts were boiled in 500 ml water for two hours and the aqueous extract was subjected to steam water evaporation for the purpose of concentration. The yield was calculated to be 5%. The extract was weighed and percentage yield was calculated in terms of air dried weight of plant material. The colour and consistency of extract were noted.

The organic solvent extraction of active constituents present in the arecanut was done by using petroleum ether, chloroform and ethanol (40% and 95%). Extracts obtained from successive solvent extraction were subjected to various chemical tests to detect the chemical constituents present in them.

Housefly larvae were collected from Pest Control Board and were maintained on wheat husks, green grams and cereals in the laboratory. *Chrysomya* flies were collected in large test tubes from Shivajinagar Beef Market, Bangalore. Identification of flies was done at veterinary hospital, Hebbal, Bangalore. Flies were reared on meat in test tubes plugged with absorbent cotton at laboratory. Larvae were obtained after 12–16 hours. *Culex* sp. was obtained from our insectary.

Larvicidal activity of the test solutions was tested by keeping the larvae in the solution containing test samples (Odyek *et al.*, 1992). This method was modified in our laboratory to test the larvicidal activity of 12 extracts against housefly and *Chrysomya megacephala* larvae. A concentration of 50 mg/ml was selected to evaluate all the extracts against both the larvae. The solutions were prepared in the form of suspension using 1% Dimethyl Sulfoxide (DMSO) in water. About 2 ml of each solution was separately taken in 100 ml beakers. 15 House fly larvae were introduced into all the beakers. Control was performed using 1% DMSO in water without test drug. All the tests were performed in triplicate.

Chrysomya larvae have a tendency to move out if the volume of the solvent is less. Hence the volume of the solvent used was 10 ml instead of 2 ml that was earlier used for house fly larvae. Except for the volume the procedure remained the same. The number of larvae died after one and two hours were counted. The larvae were considered dead if they were immobile. The percentage mortality was calculated and tabulated.

In another method the test solutions were mixed with the larval rearing media and the treatments were given through diet. The efficacy of larvicidal agent can be determined in terms of LD_{50} and LD_{90} (Odyek *et al.*, 1992; Verma and Rahman, 1984). About 2 ml of each test solution was thoroughly mixed with 10 gm of diet and 15 larvae were introduced into the diet containing test compounds in 100 ml beakers. The beakers were tightly covered with muslin cloth. Control was run similarly without test drug. All the tests were performed in triplicate. The number of larvae died after 24 h was counted. The larvae were considered dead if they were immobile. The percentage mortality was calculated and tabulated.

Larvicidal activity on *Culex* sp. was tested at five concentrations viz., 0.0312, 0.0625, 0.125, 0.25 and 0.5 mg/ml. The concentrations were prepared by diluting the stock solution in water. Test solutions were taken in 100 ml beakers containing 50 ml of test solutions separately. About 20 larvae were released into beakers containing test solution. Control was run using 50 ml of water without test material. Number of larvae died was counted after 24 h. Larvae were considered dead if they were immobile and unable to reach the water surface. The percentage mortality and LD_{50} and LD_{90} of active extracts were calculated using standard method of probit analysis (Finney, 1947).

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Table 1 shows the results of preliminary phytochemical analysis of different extracts of arecanut. Alcoholic extract contained many phytochemicals such as carbohydrates, alkaloids, glycosides, phenolics and tannins. Aqueous extract showed the presence of carbohydrates and saponins. Proteins, fixed oils and fats were absent in all extracts.

Larvicidal activity of extracts

House fly control by means of botanical extracts and plant volatiles is getting importance in recent years. The major reason for using botanicals is that they are not harmful to environment and human beings. Some investigators have reported the toxic properties of botanicals on adult and eggs of house flies. Issakul *et al.* (2002) have documented the insecticidal effect of *Mammea siamensis* crude extracts on the eggs of housefly. Sukontason *et al.* (2004) have reported that eucalyptol was very toxic to male housefly at LD_{50} of 118 μg/fly. In the present study areca nut extracts were found to be toxic to all the three test insects. The contact toxicity experiments clearly showed that *M. domestica* larvae were the most susceptible to the arecanut aqueous extract

TABLE 1. Preliminary phytochemical analysis of arecanut extracts

Extract	Phytochemicals							
	C	Alk	Gly	S	Pst	P&T	Pr.&aa	Fo&F
Petroleum ether	–	–	–	+	+	–	–	–
Chloroform	–	–	–	–	–	–	–	–
Alcoholic extract (95%)	+	+	+	–	–	+	–	–
Alcoholic extract (40%)	+	+	+	–	–	+	–	–
Aqueous	+	–	–	+	–	–	–	–

C – carbohydrates; Alk – alkaloids; Gly – glycosides; S – saponins; Pst – phytosterols; P&T – Phenolics & Tannins; Pr.&aa – protein & amino acids; Fo&F – fixed oils & fats. + present; – absent

TABLE 2. Mean percentage larvicidal activity of Arecanut aqueous extract at 50 mg/ml concentration on *Musca domestica*, *Chrysomea megacephala* and *Culex* sp. in two different assay methods

Assay method	Exposure period (in hours)	Insect		
		M. domestica	*C. megacephala*	*Culex* sp.
Contact toxicity	1	58.3 ± 48.8	13.3 ± 5.4	38.3 ± 26.3
Method	2	30 ± 38.3	54.9 ± 38.2	80.0 ± 28.2
Diet incorporation method	24	87.4 ± 21.8	67.3 ± 30.1	–

treatment and *Culex* sp. was the next susceptible insect to arecanut extracts. Except in *M. domestica*, the treatments killed more larvae of the other two insects when the exposure time was extended to 2 h (Table 2). When the extracts were incorporated in the diets of *M. domestica* and *C. megacephala* larval mortality occurred and the mortality was recorded as 87.4 and 67.3% respectively in *M. domestica* and *C. megacephala*.

Tables 3–5 show the larvicidal activity of ethanol extract of arecanut on *M. domestica*, *C. megacephala* and *Culex* sp. Six different concentrations viz., 5, 6, 7, 8, 9 and 10 mg/ml were screened against *M. domestica*; four concentrations namely 1, 2, 3 and 4 mg/ml against *C. megacephala* and five concentrations namely 0.0312, 0.0625, 0.125, 0.25 and 0.5 mg/ml against *Culex* sp. The results clearly suggested that mosquito larvae were highly susceptible to the extracts. The median lethal dose for *M. domestica* was higher (5.88 mg/ml) than *C. megacephala* (1.86 mg/ml) and *Culex* sp. (0.072 mg/ml).

The toxic property of plant extracts and plant essential oils on *M. domestica* and *C. megacephala* was already reported by some investigators. In a study Bisseleua *et al.* (2008) have found that petroleum-ether extracts of *Griffonia simplicifolia* seeds and *Zanthoxylum xanthoxyloides* roots were very toxic to the larvae of *M. domestica*. Our investigation confirms the pesticidal property of this drug by proving its larvicidal activity. Mohottalage *et al.* (2007) have reported that leaf oil of *Piper*

TABLE 3. Mean percentage larvicidal activity and lethal dose of Arecanut ethanol extract at different concentrations on *Musca domestica*

Sl. No.	Concentration (mg/ml)	Larval mortality (%)	LD_{50} (mg/ml)	LD_{90} (mg/ml)
1	5	28.8	5.88	7.9
2	6	46.6		
3	7	75.5		
4	8	88.8		
5	9	100		
6	10	100		

TABLE 4. Mean percentage larvicidal activity and lethal dose of Arecanut ethanol extract at different concentrations on *Chrysomea megacephala*

Sl. No.	Concentration (mg/ml)	Larval mortality (%)	LD_{50} (mg/ml)	LD_{90} (mg/ml)
1	1	20	1.86	4.18
2	2	48.8		
3	3	68.8		
4	4	100		

TABLE 5. Mean percentage larvicidal activity of Arecanut ethanol extract at different concentrations on *Culex* sp.

Sl. No.	Concentration (mg/ml)	Larval mortality (%)	LD_{50} (mg/ml)	LD_{90} (mg/ml)
1	0.0312	25	0.072	0.243
2	0.0625	40		
3	0.125	70		
4	0.25	90		
5	0.5	100		

betle was toxic to the adult *M. domestica* with LC_{50} of 10.3 and 8.7 mg/dm^3 after 24 and 48 hours exposure, respectively. The essential oil of betel leaves showed dose-dependent larvicidal effects on *C. megacephala* larva (Kumarasinghe *et al*., 2002). The larvicidal activity could be attributed to the terpenes and phenolic derivatives present in petroleum ether and alcohol extracts of *Areca catechu*.

In the present work *A. catechu* extracts showed larvicidal activity against house fly, *Chrysomya megacephala* and *Culex* sp. and the results are encouraging. Further works on these larvicidal agents can be performed to elucidate the mode of toxicity against the larvae.

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Identification of bug JH during post-embryonic development from red cotton bug, *Dysdercus cingulatus* (Heteroptera: Pyrrhocoridae)

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ABSTRACT: Juvenile Hormones (JHs) synthesized in the Corpora Allata (CA) of insects regulates embryonic development, repress metamorphosis induce vitellogenin synthesis and pheromone production. Several forms of JHs have been characterized from diverse Insect orders. Our earlier studies confirmed the presence and role played by JH during embryonic and post embryonic development in *Dysdercus cingulatus*. However, the present findings suggest that the CA of *D. cingulatus* secrete an active compound that is different from other known JHs. The active compound was isolated by incubating CA of *D. cingulatus* into minimal essential medium (MEM). *In vitro* products of CA were separated using thin layer chromatography (TLC).The locations were these main products migrated on the TLC did not coincide with the spots of synthetic standard of JHIII. The release of the products was shown to be CA-specific since in control incubations using the brain, no extracts of the medium in which the CA had been incubated extracted a juvenilizing; metamorphosis—inhibiting effects on final instar nymphs. The product found at an *Rf* value of about 0.5 was found to be JH active when the same was subjected for JH Activity Bioassay. Based on these results we suggest the presence of a new putative JH (Bug JH) that is different from any other known JHs in *D. cingulatus*. Chemical identification of the CA products would be the next step in this research and it is under way.

KEYWORDS: Juvenile hormone, bug JH, TLC, *Dysdercus cingulatus*, JH activity bioassay.

INTRODUCTION

Juvenile Hormones (JHs) of insects are a group of structurally related acyclic sesquiterpenoids that regulate critical physiological processes including metamorpho-

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sis and reproduction in most insect species (Davey, 2000; Gilbert *et al*., 2000; Min *et al*., 2004; Truman and Riddiford, 2007; Riddiford *et al*., 2010). Metamorphosis comprises dramatic transformation in shape and function of organs, tissues and individual cells. JH acid is an inactive precursor and metabolite of JH which actually induces cells to become competent to undergo metamorphosis, whereas ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program. The *de novo* synthesis of JH apparently is not limited to the CA since Borovsky *et al*. (1994) demonstrated the synthesis of JH III from acetate by the ovaries of *Aedes aegypti*. Accessory sex glands of *Hyalophora cecropia* (Shirk *et al*., 1983) and imaginal disks of *Manduca sexta* (Spargana *et al*., 1985) also are known to synthesize JH II and I. JH III has also been identified in the sedge, *Cyperus iria* and suggested that this compound may have a role in protection of the plant against insect herbivory (Bede *et al*., 1999a) or may function as an allelopathic agent (Bede and Tobe, 2001). The three compounds JH I, JH II and JH III differ widely in their physiological activity, as well as in haemolymph concentration during the insectan life cycle. Roller *et al*. (1967) first determined the chemical structure of Juvenile Hormone. The hormone proved to be an unusual sesquiterpenoid with an epoxide group near one end and a methyl ester on the other. The hormone that was identified soon proved to be one of a series of naturally occurring JH, all of which have been nearly identified their chemical structures, differing only in the number of ethyl side chains. Roller's hormone is now named as JH-I ($C_{18}H_{30}O_3$) the other two are called JH-II $C_{17}H_{28}O_3$ (Meyer *et al*., 1968) and JH-III $C_{16}H_{26}O_3$ (Bowers, 1969). JH 0 and its isomer, 4-methyl JH I (Iso JH) were identified in *M. sexta* eggs (Bergot *et al*., 1981) controls the early embryonic development. In the Cyclorrhaphous dipterans like *Drosophila melanogaster, Calliphora vomitoria, Phormia regina and Lucilia cuprina* a new JH homologue, the bis-epoxide of JH III, was identified (Richard *et al*., 1989; Yin *et al*., 1995). An additional hydrolated JH, 4′-OH-JH III was identified in locust CA *in vitro*, that opened a new perspective for the studies on JHs (Mauchamp *et al*., 1999).

The three compounds JH I, JH II and JH III differ widely in their physiological activity, as well as in haemolymph concentration during the insectan life cycle. JH III is the predominant hormone in most adult insects, while JH I, JH II having been identified mainly in the larvae and nymphs of many insect orders. This suggests that JH I and JH II are morphogenetic, whereas JH III is gonadotropic (Lanzrein *et al*., 1978). Some insects secrete only one of the three forms of JH; while others secrete a mixture of two or all the three of them. JH-III is the only form found in Orthoptera. The principal form of JH found in Coleoptera, Diptera, Hemiptera and Hymenoptera is JH-III only. The Lepidoptera appears to be unique in secreting a mixture of JH-I and JH-II. JHO and 4-methyl JH I is found only in the eggs of Lepidoptera (Nijhout, 1994; Davey, 2000).

However, the chemical identity of JH in Heteroptera is not settled yet. In a pyrrohocorid bug, *Dysdercus fasciatus*, the product of the CA *in vitro* was shown to be methyl farnosoate by Feldlaufer *et al*. (1982) while Bowers *et al*. (1983) using a

different incubation medium, indicated that CA of this species a ligaeid, *Oncopeltus fasciatus* and a pentatomoid, *Nezara viridula* released JH III *in vitro*. Our earlier studies established the presence and role played by JH during embryonic and post embryonic development in *Dysdercus cingulatus* (Gayathri and Muraleedharan, 2001, 2007). The present study is accomplished to address the question whether the adults of *Dysdercus cingulatus* do secrete a different form of JH.

MATERIALS AND METHODS

Insect

The red cotton bug *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae) was used for the study. The method for rearing bugs has been described previously (Gayathri and Muraleedharan, 2001).

Incubation of Corpora allata (CA)

The CA was taken out from reproductively active females of *D. cingulatus* under a stereomicroscope and incubated in the minimum essential medium (MEM). After an incubation period of 3 h at 30°C, the incubation medium was extracted (Kotaki, 1996).

Thin layer chromatography

The Hexane–extracts thus obtained were applied on a TLC sheet aluminium gel 60 F_{254} (MERCK) along with synthetic standards of JH III (Sigma USA). The TLC sheet was developed using Hexane and Ethyl acetate in 1:2 ratio and spots of Standards were visualized under a UV-light. To determine the JH active fraction, hexane–extracts of the MEM in which the CA had been incubated for 5–6 h were subjected to TLC separation as above.

JH activity bioassay

To test the JH activity of products of CA of *D. cingulatus* an aliquot the hexane–extracts was topically applied to fifth instar nymphs of this bug (0-day, 2-day, 4-day old).

Quantification of total protein

Protein concentration was determined by the Coomassie Brilliant Blue dye binding method of Bradford (1976).

Tris glycine SDS-PAGE

The electrophorectic protein profile of haemolymph and fat body of final instar nymphs which had undergone JH activity bioassay were determined by one-dimensional Sodium-Dodecyl-Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) carried out according to Laemmli (1970) under discontinuous and dissociating buffer systems.

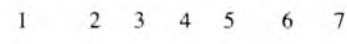

FIGURE 1. TLC separation to confirm Products are CA specific. Lane 1: JH III, Lane 2: Brain, Lane 3: CA products, Lane 4: midgut Lane 5: gut Lane 6: Muscle; Lane 7: Fat body.

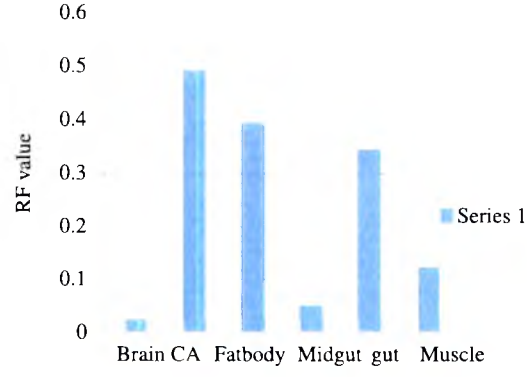

FIGURE 2. TLC analysis to confirm products are CA specific.

RESULTS

To determine the JH active fractions separated by TLC eluates from TLC with ethylacetate were concentrated redissolved in hexane and applied to final instar nymphs. Spots of various *Rf* values were used for JH activity Bioassay. The CA from reproductively active females of *D. cingulatus* released products into the incubation medium which did not co migrated with the synthetic standard of JH III (Figs 1 and 2). Control incubations of the brain, midgut, a piece of fat body, muscle indicated that these products were CA specific. Spots of various *Rf* values were scrapped out and used for JH activity bioassay. When spots corresponding to *Rf* value of 0.5 (six individual CA equivalents) was applied on to just moulted 5th instar nymphs they emerged as supernumerary nymphs. The deformities observed in nymphs treated with the extracts of six individual CA equivalents were significantly higher than those nymphs that received lower doses (4 CA equivalents and those of controls). No JH activity was found in the sub fractions at *Rf* values of 0.2 to 0.4 and that of R*f* values of 0.56–0.6.

FIGURE 3. Insects after JH activity Bioassay. I. *D. cingulatus* adult female, II. *D. cingulatus* 5th instar nymph, III. supernumerary nymph, IV. Adultoid.

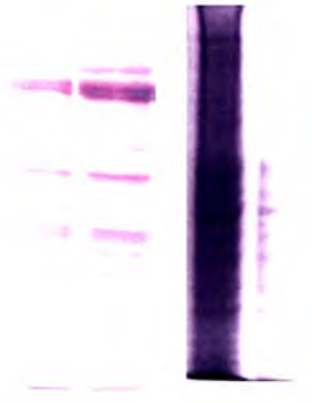

FIGURE 4. Electropherogram showing fatbody and haemolymph protein profile of JH active Hexane extract received 5th instar nymphs of *D. cingulatus*. Lane 1: Heamolymph from treated nymphs, Lane 2: Control haemolymph Lane 3: Fatbody from treated nymphs, Lane 4: Control fatbody.

The supernumerary nymphs and the adultoids retained varying degrees of nymphal characters (Fig. 3). Concentration of total haemolymph proteins in control and treated *D. cingulatus* nymphs of various age groups revealed a reduction in total protein. Significant reduction in the intensity and the number protein bands in both fat body and haemolymph of JH-treated nymphs noticed when compared to that in control adults (Figs 4 and 5).

DISCUSSION

Juvenile hormone is of decisive importance in the regulation of development, metamorphosis and reproduction in insects. Juvenile hormone act as unique repressor agent which inhibit morphogenetic process for a determined time at any stage between the fertilized egg cell and fully differentiated adult and is hence responsible for the existence of polymorphic immature stages so characteristic of insect development. The

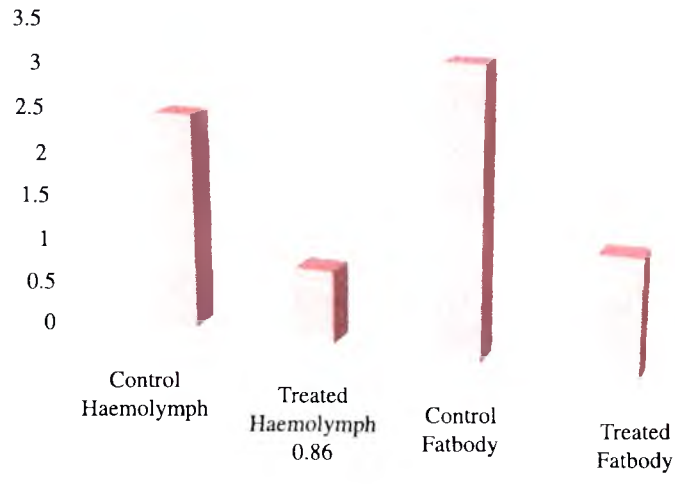

FIGURE 5. Total protein profile of JH active extract received 5th instar nymphs of *D. cingulatus*.

results signify that the CA of Red Cotton Bug *D. cingulatus* release JH active products *in vitro*. None of these products perform in the same way to JH III. TLC separation of the hexane extracts followed by topical application of the eluates from the TLC cut parts to final instar nymphs reveals that has JH activity. Electrophorectic protein profile studies were done with haemolymph and fat bodies of *D. cingulatus* nymphs that received JH active hexane extracts. These studies showed there is a reduction in both fat body and haemolymph protein levels similar to the treatment with Juvenile hormone analogue Methoprene (Gayathri and Muraleedharan, 2007).

The CA of the Tobacco Hornworm, *Manduca sexta*, have been accounted to biosynthesize an unidentified product which yields JH III-acid after ester hydrolyses (Granger *et al.*, 1995). In several insects, it has been known that the CA produce and release JH-related substances without JH activity. The CA of *L. migratoria* produce JH III diol in addition to JH III (Gadot and Goldman, 1987). In several lepidopterans species, the CA of male adults release JH acids (Cusson *et al.*, 1999). Ho *et al.* (1995) have identified iso –JH -acid as a product *in vitro* by the CA of the male adults in *L. loreyi*.

The *Rf* value of the foremost products on the TLC is different from that of JH III. These results robustly propose that the fraction corresponding to the *Rf* value 0.5 that shows JH activity in *D. cingulatus* is not the same as known JHs but a new, unidentified JH. It is also shown that the CA of some other pentatomid and alydid bugs also biosynthesize a product with an *Rf* value similar to that of the major CA product by *P. stali* (Kotaki, 1993, 1997). Therefore, Heteropterans or at least some species in Pentatomidae and Alydidae may have a common new JH.

The structure of the juvenile hormone (JH) in the suborder Heteroptera, order Hemiptera, has been known for a very long time to be different from the JH of other

orders, but the structure has been a matter of controversy. The structure was first elucidated by an unprecedented approach involving the screening of a JH molecular library. The novel Heteroptera-specific JH (JHSB(3)) is a new category of JH that is featured by the skipped bisepoxide structure (Kotaki *et al.*, 2009).

Chemical identification of the putative JH active CA product in the Red cotton bug *D. cingulatus* would be the subsequent step of this research, and it is on the move in our laboratory.

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Revision of the rare oriental genus *Sepsidoscinis* Hendel (Diptera: Chloropidae: Oscinellinae: Elachipterini) with description of a new species from India

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ABSTRACT: A new species of *Sepsidoscinis* Hendel from India is described and some characters of the genus not reported earlier and key to species are given.

KEYWORDS: Chloropidae: Oscinellinae: Elachipterini: *Sepsidoscinis nigra*, sp. n., India.

Nartshuk (1983, 1987) in her revisionary works on the family Chloropidae erected the tribe Elachipterini. She included under the tribe 10 genera namely, *Anatrichus* Loew, *Disciphus* Becker, *Elachiptera* Macquart, *Melanochaeta* Bezzi, *Myrmecosepsis* Kertesz, *Sepsidoscinis* Hendel, *Alombus* Becker, *Ceratobarys* Coquillett, *Cyrtomomyia* Becker and *Togeciphus* Nishijima of which the first 6 were earlier placed by Andersson (1977) under his *Elachiptera* genus group. She removed *Cadrema* Walker from *Elachiptera* genus group and added *Togeciphus* Nishijima to the group as suggested by Kanmiya (1983). Of these genera Wheeler and Forrest (2002) synonymised *Ceratobarys* with *Elachiptera*. Mlynarek (2009) in her work on the tribe (not published but available online) synonymised *Togeciphus* and *Cyrtomomyia* with *Elachiptera* and *Sepsidoscinis* with *Anatrichus* but recognized *Ceratobarys* a distinct genus. Later, Mlynarek and Wheeler (2010) added *Goniaspis* Duda, as suggested earlier by Sabrosky (1984) in his unpublished manuscript, and *Allomedeia* Mlynarek and Wheeler to the tribe, thereby recognizing only 9 genera under Elachipterini. However, Nartshuk (2010) included all the 10 genera, including *Togeciphus*, *Cyrtomomyia* and *Sepsidoscinis*, placed earlier by her under the tribe. But she left out *Alombus* from her list of genera without clarification and expressed her reservations in placing *Melanochaeta* Under Elachipterini.

Cherian (2011) added the genera *Parameijerella* Cherian (1991) and *Melanochaetomyia* Cherian (2002), which are typical members of Elachipterini, to the group and

recognized 14 genera, including *Alombus*, under the tribe. He also gave a key to the genera of the tribe and provided clarifications for the resurrection and placement of some of the genera under the tribe.

Sepsidoscinis Hendel is a small genus known only by the type species *S. maculipennis* Hendel from the Oriental Region. A new species, *S. nigra* from Kerala, India is described here. Besides, the genus is redefined incorporating some characters not reported earlier and a key to the species of the genus is also given.

The type specimen is retained at present in the collections of the Department of Zoology, University of Kerala, Trivandrum and shall be deposited later in the National collections of the Western Ghats Regional Centre, Zoological Survey of India, Kozhikode (Calicut).

Sepsidoscinis Hendel

Sepsidoscinis Hendel, 1914. *Ann. hist. nat. Mus. natn. Hung.*, *12*:247.

Type species: *S. maculipennis* Hendel. By original designation.

Generic characters:

Head dorsoventrally compressed, longer than high and swollen posteriorly; frons longer than wide, narrowing interiorly; frontal triangle large, reaching anterior margin of frons; face short with or without facial carina; arista long, slender with fine pubescence; gena narrow; eye with horizontal to oblique long axis and fine scattered pubescence; *ovt* subequal to or shorter than *ivt*, the latter at times conspicuously proclinate; *oc* proclinate; prothorax prolonged, neck-like with lateral transverse ridges Scutum greatly convex; scutellum thick, rounded along lower margin and triangular to conical dorsally, with 4 tuberculate bristles; *npl* 0+1 or 1+1; wing much shorter than body with dark transverse bands; costal break absent; legs long and slender with small tibial but no femoral organ; abdomen clavate with two petiolate basal segments; surstylus large, bifid; hypandrium open with anterolateral projections.

Distribution: Oriental Region.

Remarks: Among the various genera of tribe Elachipterini, only in *Sepsidoscinis* Hendel and *Myrmecosepsis* Becker and *Alombus* Becker, head is dorsoventrally compressed and longer than high. But unlike in *Alombus* and other genera of the tribe, in *Sepsidosicinis* and *Myrmecosepsis* prothorax is prolonged, neck-like and with transverse lateral ridges, costal break at or before ending of R 1 is absent and postgonites are linear, oblong and not triangular distally. The absence of a costal break is a unique character not reported so far in any other genus of subfamily Oscinellinae.

The combination of characters in the two genera had prompted Kanmiya (1983) to consider them belonging to a separate genus group.

In *Myrmrcosepsis taprobane* Andersson (1977) there is a long well developed proclinate and a little lateroclinate bristle which Andersson considered of "uncertain identity" as only one of the vertical bristle is present in the species. But Kanmiya

(1983) and later workers considered it representing *ovt*. However in both the species of *Sepsidoscinis*, including a new species described here, *ovt* and *ivt* are present of which the latter is more developed and in the new species very prominent and distinctly proclinate as in *M. taprobane*. Because of the position of this bristle it is apparent that it represents *ivt* and not *ovt*, the latter relatively weaker in *Sepsidoscinis* and virtually not developed in *Myrmecosepsis*. Hence the development of a very long, well developed and proclinate *ivt* is considered here a character both the genera have in common along with other characters, especially the important synapomorphic character of the absence of the costal break.

This genus is so far known by the type species *S. maculipennis* which is widely distributed in the Oriental Region, including India. A new species, *S. nigra* from India is described here which is the second species coming under the genus. A key to both the species is also given.

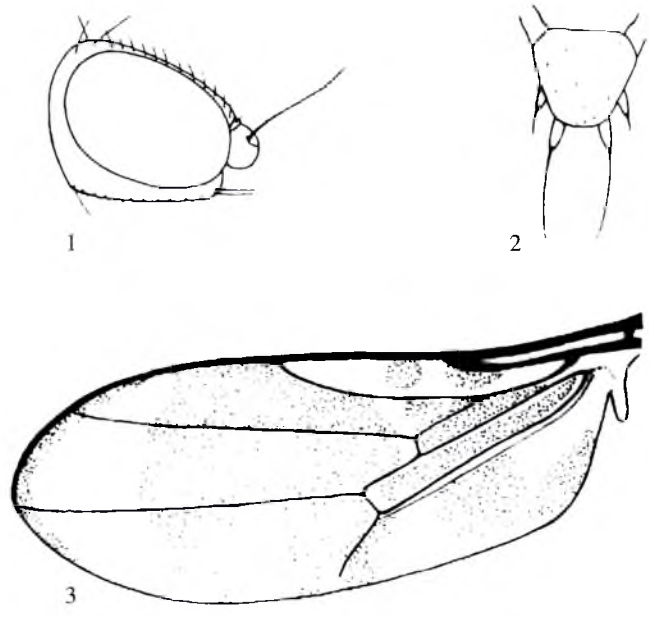
Key to species of *Sepsidoscinis* Hendel

Facial carina indistinct; *ovt* and *ivt* subequal, the latter proclinate and lateroclinate; frontal triangle without midlongitudinal stria; *if* in a row on lateral margin of frontal triangle; scutum entirely brown; *npl* 1+1; *ss* 1 a trifle longer than the tubercle on which it is borne ..... *maculipennis* Hendel

Facial carina developed, running as a low narrow ridge to almost epistomal margin; *ovt* shorter than i*vt*, the latter prominent and distinctly proclinate as in species of *Myrmecosepsis*; frontal triangle with linear shallow midlongitudinal stria; *if* in a row on frontal triangle inside each lateral margin; scutum yellowish brown anteriorly and brownish black in the middle and distal half; *ss* 1 shorter than the tubercle on which it is borne ..... *nigra*, sp. n.

***Sepsidoscinis nigra*, sp. n. (Figs. 1–3, Pl. 1)**

Male: Head (Fig. 1): Head longer than high, height, length and width ratio 19:21:24, swollen behind and narrowing in front. Frons extending behind eye margin posteriorly but not reaching anterior eye margin, smooth, narrowing and finely tomentose anteriorly, width at vertex 0.63× that of head, 0.83× its own length and 1.5× its width near anterior margin; frontal triangle large, at vertex only a little narrower than frons, with slightly convex lateral margins, smooth shiny yellowish brown with light dark tinge in certain angles of illumination, finely tomentose along sides and around apex and with midlongitudinal linear shallow stria running from anterior ocellus to a little behind its anterior margin, reaching anterior margin of frons and ending with narrowly obtuse apex. Face short and narrow, finely tomentose; facial carina, unlike in *maculipennis*, triangular between bases of antennae and running as a low narrow ridge almost to epistoma which is broadly conical and projects forward. Antenna brownish; *ant* 2 small; *ant* 3 suborbicular, wider than long with fine pubescence; arista long, slender with short pubescence. Gena narrow, about 0.3× as wide as *ant* 3, pale yellow with a row of many oral setae and a few hairs especially



FIGURES 1-3. *Sepsidoscinis nigra,* sp. n. 1. Head – lateral view. 2. Scutellum – dorsal view 3. Wing

PLATE I. *Sepsidoscinis nigra,* sp. n (Male).

in lower half; vibrissal corner almost reaching anterior margin of eye, rounded with 2 subequal vibrissae; postgena broad, well developed as in *maculipennis* with hairs arranged in one to two rows; parafacialia not developed. Palpi yellowish brown; proboscis with long slender labellum; occiput and adjoining area well developed. Head bristles mostly black but *oc, orb* and *if* pale; *ovt* shorter than *ivt*, the latter prominent

and distinctly proclinate as in species of *Myrmecosepsis* and unlike in *maculipennis* in which *ovt* and *ivt* are subequal and the latter is proclinate and lateroclinate; *pvt* erect, cruciate, subequal to *ovt*; *oc* proclinate, nearly parallel, shorter than *if*; *orb* in a row of 11 to 12, reclinate; *if* about 7 to 8, in a row distinctly on the triangle inside each lateral margin unlike in *maculipennis* in which *if* are arranged on margin of triangle.

Thorax: Prothorax prolonged, neck-like with transverse ridges and a pair of short, blunt protuberances on each side. Humeral callus though reportedly not distinct in *maculipennis* is discernable below one of the projections on each side of neck as evidenced by a distinct *h*1 bristle stated to be absent in the genus. Scutum narrowing anteriorly and greatly convex medially, shiny yellowish brown with diffused dark tinge anteriorly and gradually becoming entirely brownish black medially and posteriorly including sides unlike in *maculipennis* in which scutum is entirely shiny brown and without spots; length of scutum including neck and width ratio 13:10; hairs on scutum long, slender, evenly distributed, black but appearing pale in some angles of illumination. Pleura shiny dark brown with black, diffused maculae on lower part of *kepst* and *anepm*; *anepst* without and *kepst* with a few hairs. Scutellum (Fig. 2) wider than long, tomentose, thickened, conical, flattened and yellow on dorsum and brownish black and rounded along sides and lower margin, with black hairs on dorsum and sides especially distally. Thoracic bristles black; *h*1 developed, shorter and slender than *npl*; *npl* 0+1, well developed; *pa* 1 and *pa* 2 hardly distinguishable from thoracic hairs; 1 *dc* developed, shorter than *npl*; *as* widely separated at base, more than 2× as long as the prominent tubercle on which it is borne; *ss* 1 a little shorter than its basal pale prominent tubercle which is shorter than the tubercle at base of *as*; scutellar tubercles borne on lower margin of scutellum.

Wing (Fig. 3): Long and narrow, more narrowing basally, 2.7× as long as wide with three transverse bands of which median is large, covers median part of wing and is more deeply darkened in upper half, distal one along apex of wing is very short and narrow and is only diffused brown and basal one is diffused dark brown with the colouration restricted to some areas; costal break near apex of R 1 absent on in *maculipennis*; proportions of costal sectors 2 to 4 in the ratio 11:16:9; *r–m* cross-vein far distad of middle of discal cell, opposite 0.7 of its length; running very close to and extending to more than three-fourths basal sector of M 3+4 and adjacent to discal cell a distinct vein-like fold is present as in *maculipennis*; anal area receding. Stalk of haltere dark brown, knob pale yellow with dark tinge at base.

Legs: Long and slender; all coxae and trochanters yellow; hind leg fallen off; fore and mid femora yellow with the former weakly and the latter more prominently with diffused deep dark tinge in distal halves dorsally; tibiae yellow with fore tibia weakly and mid tibia almost wholly with dark tinge; fore and mid tarsi yellow; femoral organ absent.

Abdomen: Wholly black with diffused dark brown tinge in some areas, finely tomentose and with a few slender conspicuous hairs especially on distal segments; two basal segments long, narrow and petiolate, their combined length subequal to length of succeeding segments together; abdomen bends down at base of third segment, turns forwards, becomes large oval and clavate and is kept below two basal segments; third tergum much longer than the fourth; segments from five becomes narrower than preceding segments.

Length: Male: 4.9 mm; wing: 2.8 mm.

Holotype: Male, India: Kerala: Trivandrum Dist., Veli, 20 m, 18.xi.2007, Coll. A. K. Shinimol.

Etymology: The species derives its name from the black colour of its scutum.

Remarks: *S. nigra* shows close affinities to *maculipennis* Hendel from the Oriental Region, the only species of *Sepsidoscinis* so far known. However it differs from *maculipennis* in *if* being arranged on frontal triangle distinctly inside each lateral margin, in having proclinate *ivt* which is more developed than *ovt*, a midlongitudinal linear stria on frontal triangle, fairly well developed facial carina and posteriorly brownish black scutum. But in *maculipennis if* are arranged along margin of frontal triangle, *ivt* is subequal to *ovt* and is not proclinate, midlongitudinal stria on frontal triangle is absent, facial carina is indistinct and scutum is wholly brown and without spots.

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Abbreviations

anepm – anepimeron; *anepst* – anepisternum; *ant* 2 – second antennal segment; *ant* 3 – third antennal segment; *as* – apical scutellar bristle; 1 *dc* – first dorsocentral bristle; *fr* – frontal hair; *h* – humeral bristle; *if* – interfrontal bristle; *ivt* – inner vertical bristle; *kepst* – katepisternum; *npl* – notopleural bristle; *oc* – ocellar bristle; *orb* – fronto-orbital bristle; *ovt* – outer vertical bristle; *pa* –postalar bristle; *pvt* – postvertical bristle; *ss* – subapical scutellar bristle.

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Mite feeding wounds on honey bee pupa-morphoarchitectural study

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ABSTRACT: Studies were conducted on *Varroa* infested and non-infested (control) worker brood of the European honey bee *A. mellifera*. The structure of punctures and wounds on the pupae of *A. mellifera* infested with mite by SEM analysis. The preferred sites of the mites on the worker pupa were the anterior sternites of the abdominal segments and a lateral position on the thorax. SEM studies clearly revealed spherical and/or irregular punctures and the surrounding area was distinctly scarred. Details are illustrated in the paper.

KEYWORDS: *Varroa*, *Apis mellifera*, worker pupae, SEM

INTRODUCTION

Like all living beings, the honey bee is subject to be attacked at all stages of its development by various enemies acting directly as predators, or indirectly by disturbing the life of the colony in various ways. The enemies of bees can be classified as parasites, predators, or commensals, depending on the nature of their interaction with bees.

Mites (Acari) found in the honey bee colony may be feeding only on pollen, phoretic – using the bees for hitch hiking, parasitic – both ecto and endo. Important among the latter are *Acarapis woodi*, *Varroa destructor* and *Tropilaelaps clareae*. The problem of spread of bee mites is enhanced by the fact that these have great dispersal potential being carried first by their host and then by man who moves bee colonies for commercial gains and pollination services. This has been witnessed in the rapid spread of *Varroa* to different countries of the world.

Ectoparasitic mites of the genus *Varroa* are known from Asian honey bees, of which nine extant *Apis* species are recognised (Koeniger and Koeniger, 2000). All life stages of *Varroa* mites feed exclusively on bee haemolymph after perforating the host's integument with their chelicerae (Smirnow, 1979; Donzé, and Guerin, 1994).

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In new host the European honey bee (*A. mellifera*) the *Varroa* ectoparasitic mites cause serious health disorders collectively referred to as Varroasis (De Jong, 1997). Today varroasis is the main problem for beekeeping with *A. mellifera*. *V. destructor* is a taxon recently separated from *Varroa jacobsoni* Oud. (Anderson and Trueman, 2000). The life cycle of *Varroa* can be divided into a phoretic phase, with female mites only attached to adult bees, and a reproductive phase which is spent in capped bee brood cells containing a last instar, which female mites invade shortly before they are sealed (Ifantidis and Rosenkranz, 1988). In the still open brood cell, honey bee workers deposit a large portion of larval food into which the invading *Varroa* female submerges (Ifantidis, 1988). After capping of the cell, these provisions are eaten by the bee larva within about 5 h. The parasite immediately begins to suck the bee haemolymph (Steiner *et al.*, 1994). *Varroa* wounds in the host can be visualised by vital staining with trypan blue (Kanbar and Engels, 2004). Due to repeated feeding of the adult and nymphal mites, the healing of the perforation is prevented until scarring occurs prior to the imaginal moult.

The aim of the present studies was to investigate in detail the structure of punctures and the wounds on the host pupae of *A. mellifera* infested with mite by SEM analysis.

MATERIAL AND METHODS

Present studies were conducted on *Varroa* infested and non-infested (control) worker brood of the European honey bee *A. mellifera*.

Study area

The samples of *A. mellifera* worker brood were drawn from the colonies maintained by the department of Zoology, Panjab University, Chandigarh.

Study material

A. mellifera worker brood was taken for investigations. Kumar *et al.* (1993) on the basis of studies on the mite infestation of honey bee brood had suggested that the late pupa (16–20 days old) is the most vulnerable stage in the life cycle of host. Worker brood samples, infested and non-infested were drawn at this stage. The late pupa can easily be distinguished by clear body division into head, thorax and abdomen, well formed appendages and brown eye colour.

Sample collection

A random sample of 10 infested and 10 non-infested worker pupae (brown eye stage) was taken for each test after brushing off the bees from the comb.

SEM studies

For SEM studies of the specimens, the following standard protocol was adopted. The collected material of infested and non-infested pupae of *A. mellifera* was fixed in

FIGURE 1. Uninfested pupa (Dorsal view).

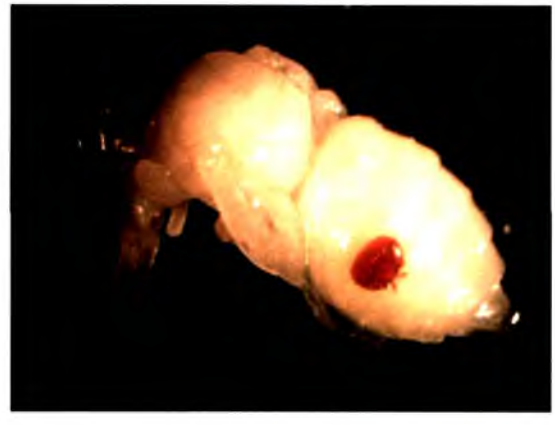

FIGURE 2. Infested pupa with mite on abdominal region.

4% glutaraldehyde. The preserved material was then washed with phosphate buffer, dehydrated in graded series of acetone followed by amyl acetate and critical point drying.

Mounting and sputtering

Dehydrated samples were mounted on the stubs in the desired orientation with the help of double side adhesive tape under binocular microscope. The samples were attached in such a way that they remained visible from all the sides. Stubs were then placed inside the sputter for gold coating to overcome the problem of "charging" and "beam damage".

FIGURE 3. Uninfested pupa showing thoracic region with no lesions.

FIGURE 4. Thoracic region showing dermal aberrations made by the mite.

Scanning

The sputtered specimens were examined in Jeol JSM-6100 scanning electron microscope operating at an accelerating voltage of 10 KV at regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh and the ultra structural differences were studied.

RESULTS

Scanning electron micrography revealed healthy integument of the non-infested pupa (I). There were no punctures or wounds visible on the body of non-infested pupa (III). The site of mite feeding on the thorax of infested pupa was aberrated (IV). The infested pupa on the other hand had distinct spherical to oval punctures on the abdominal

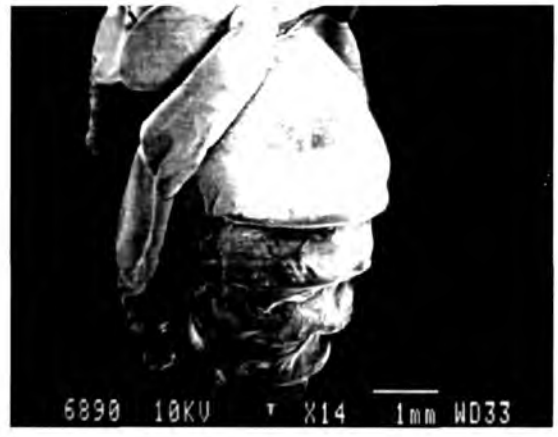

FIGURE 5. Uninfested pupa showing abdominal region without any puncture.

FIGURE 6. Puncture made by the mite on region of infested pupa.

sclerite and lesions at the sites of mite injury (VI). The adjoining area showed irregular epidermis and lesions (VII, VIII). Wound area with granulation tissue was evident both in the thorax and abdomen at the site of mite injury.The lesions presented an extensively criss-cross pattern characteristics of granulation tissues in scarred regions near the feeding sites of the mite (Fig. 9).

DISCUSSION

Apis mellifera is a new host of the *Varroa* mite. This host shift was the result of contact of the European species with Asian honey bees as a consequence of its introduction in several parts of Asia. The change proved more damaging for this species because of the ability of the mite to reproduce successfully in both the worker

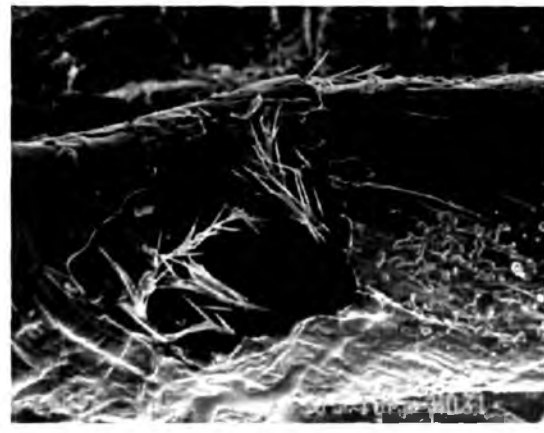

FIGURE 7. Puncture alongwith local lesions on the feeding site of the host pupa.

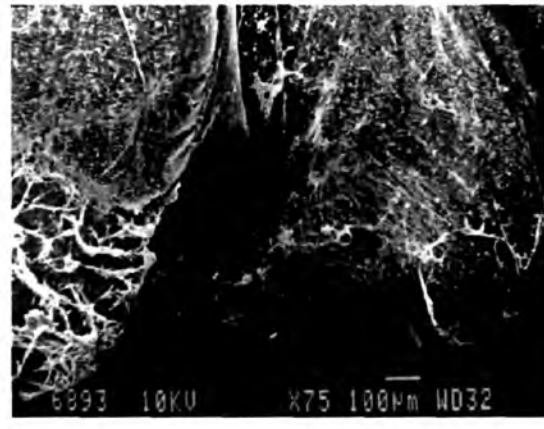

FIGURE 8. Perforation made by the mite (Magnified view).

brood and drone brood, unlike the indigenenous host *A. cerana* where it preferentially parasitizes the drone brood. The parasite was found adhering to the thorax as well as to the intersegmental region between the abdominal sclerite on the worker pupa. Punctures and/or lesions were observed on two specific locations on the host viz the lateral thoracic region and the intersegmental region of the anterior abdominal sternites. Kanbar and Engels (2003) also made similar observations though they also observed wounds on the sixth sternite region. SEM revealed characteristic oval punctures surrounded by an aberrated wound area, lesion and granulation were also distinct in this region. Kanbar and Engels (2004) had used Trypan blue to localize the damaged cells in the integument of infested infested host. The scarring/aberrations

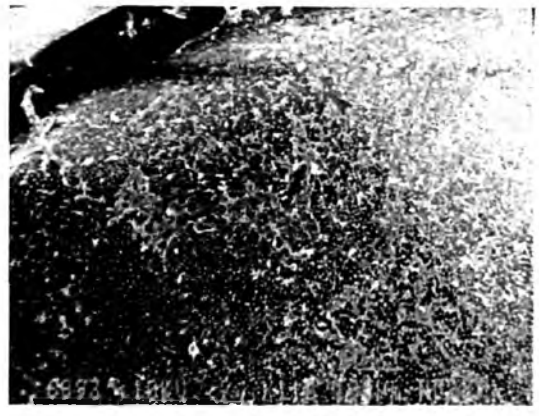

FIGURE 9. A scarred wound area.

observed in the present study were probably due to the activity of mite chelicerae as also suggested by these authors (Kanbar and Engels, 2003).

ACKNOWLEDGEMENTS

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Diversity of *Aedes* larval habitats in rural and urban areas of Malappuram District

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ABSTRACT: Entomological surveillance was carried out in randomly selected rural and urban areas of Malappuram District to find out the breeding habitats, larval indices, preferred breeding habitats and seasonal variation of *Aedes* mosquitoes. The Aedes larval indices such as House index, Container index and Breteau index were significantly higher in monsoon than that of pre-monsoon in both rural and urban areas of Malappuram district. In rural area the most preferred breeding sources of Aedes mosquitoes based on Breeding Preference ratio (BPR) were more in coconut shells (1.26) followed by metal (1.25), plastic (1.23) and mud containers (1.06). The habitat preference of dengue fever vector mosquitoes in urban situations showed that BPR was more in metal containers (1.34) followed by plastic (1.24), mud containers (1.22) and coconut shells (1.19).

KEY WORDS: *Aedes*, diversity, breeding preference

INTRODUCTION

Vector-borne diseases are among the major causes of human sufferings in terms of morbidity and mortality, on one hand, and stunting the social and economic growth of the countryon the other. Dengue is one of the most serious and fast emerging tropical diseases among all the arthropod-borne viral diseases (Gubler and Kuno, 1997). Since fifties dengue virus activity was known to occur in Kerala (Banerjee and Desai,1973). However, dengue epidemics have been reported from 1997 onwards. A total of 1,1792 DF/DHF cases with 68 deaths (0.58%) were reported from Kerala during 2006-2012. Dengue fever has become almost perennial in some districts of the State. DF is comparatively higher In Thiruvananthapuram, Kottayam, Idukki, Kannur and Kasargod Districts (DHS.2010).

Dengue fever cases have been reported from Malappuram district since 2002. A total of 235 DF cases with one death (0.43 %) were reported from Malappuram district from 2006–2012. Chikungunya fever appeared as epidemic in 2006, added

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a new dimension to the entire scenario of vector-borne diseases in Kerala. The disease was reported largely from South Kerala in 2006, middle Kerala in 2007 and northern part in 2008. In 2009 chikungunya largely affected Kozhikode, Malappuram, Kannur and Kasargod districts. A total of 6252 suspected and 194 serologically confirmed chikungunya cases were reported from Malappuram district during 2006–2012.

In Asia, *Ae. aegypti* is considered to be the principal vector of DF/DHF followed by *Ae. albopictus*, which assumes importance in some area and, together with *Ae. aegypti*, can be involved in serious outbreaks of DHF (WHO, 1993). *Aedes* mosquitoes are known vectors of dengue fever and chikungunya. The knowledge regarding the prevalence, breeding habitats and seasonal variation of *Aedes* mosquitoes in Kerala is meager. Hence, a study has been carried out on the bio-ecology of *Aedes* mosquitoes in rural and urban areas of Malappuram district.

MATERIALS AND METHODS

Study area

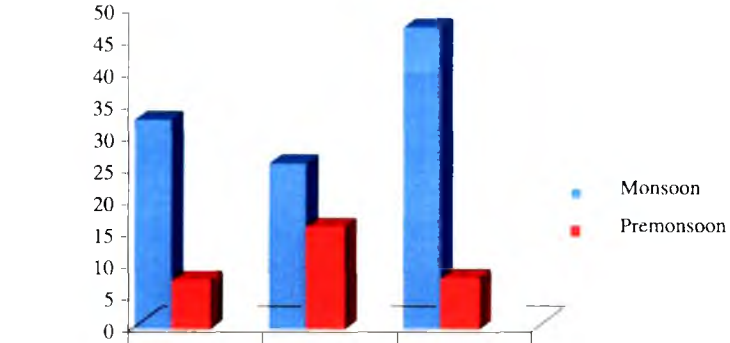
Malappuram is the southernmost of the northern districts of Kerala. It shares boundaries with Kozhikode and Wayanad in the North and Tamil Nadu in the East. Palakkad forms most of its East and South boundary with Thrissur. Malappuram district lies between 11°-1′ and 48″ North latitude and between 76°-3′ and 0″ East longitude.

Entomological study was carried out in randomly selected rural Wards, namely, WardNo. 2 of Anakkayam and Ward No. 6 of Thrikalangod Panchayats and urban Wards, namely, WardNos.16 &14 of Manjeri Municipality in MalappuramDistrict. The type of monitoring was regular fortnightly. The houses and their surroundings were searched for mosquito larval breeding during June 2006 to May 2007. All the water holding containers/habitats were checked for larval breeding and recorded. Larvae/ pupae from each positive container were collected separately. Fifty percent of the larvae were identified microscopically and the remaining larvae and pupae were identified after adult emergence following WHO guidelines (WHO.1995). *Aedes* larval indices- House index (HI), Container index (CI), Breteau index (BI), the preferred breeding habitats and seasonal variation of vector breeding were analyzed. Adults of *Aedes* mosquitoes were also collected while landing on human baits by aspirator tubes and Per Man Hour (PMH) density was calculated as per standard procedure.

Statistical analysis was done with SPSS-version 18. Kolmogorov- Smirnov test was done to test the normality of data, Levene's test for equality of variances and independent sample t-test for equality of means.

RESULTS

A total of 1440 house premises were searched for *Aedes* breeding in Wards of Anakkayam and Thrikalangode Panchayats. *Aedes* breeding was recorded in 337 premises (HI-23.40%). In order to assess the container/habitat preference of *Aedes* mosquitoes the breeding sources of the peridomestic areas were conveniently classified as 1) man-made/ artificial and 2) natural. Of the total 1802 water holding



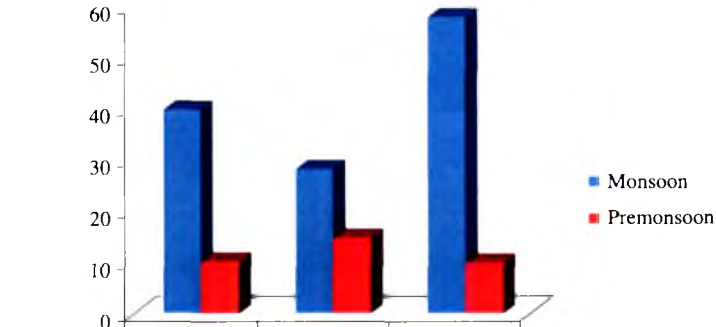
	HI	CI	BI
Monsoon	33.1	26.3	47.6
Premonsoon	8	16.4	8.3

FIGURE 1. *Aedes* larval indices in monsoon and pre monsoon (Rural area)

containers/ sources searched from rural area, 496 (CI-27.52%) were positive for *Aedes* larvae. In order to find out a relation between positive containers and number of houses examined for larval presence, the Breteau index (BI) was calculated and was found to be 36.39 in rural areas. It is also found from the present study that *Ae. albopictus* has been the predominant species (98.69%) and *Ae. vittatus* larvae constituted only 1.31%.

In Kerala, the South-west monsoon starts in the beginning of June and fades out by September. The North-West monsoon commencing in October continues up to mid December. The summer starts in the middle of January and continues till May. An attempt was made to compare the *Aedes* larval indices in monsoon and pre monsoon. In rural area the mean score of HI , CI and BI during monsoon were 33.1 ± 2.9, 26.3 ± 1.5 and 47.6 ± 6.9 respectively (Fig. 1). In pre monsoon the *Aedes* larval indices were low comparing monsoon months (HI-8.0 ± 1.9, CI-16.4 ± 1.3 and BI- 8.3 ± 1.2). The normality of the scores were tested by Kolmogorov-smirnov test and the result showed that the data are normal ($p>0.05$). Also, the Levene's Test for Equality of Variances confirmed that the variances are equal in between monsoon and pre monsoon for the indices, HI,CI and BI ($p>0.05$). The t-test showed that the mean scores in monsoon are significantly higher than that in pre monsoon for the indices HI ($t = 6.52$, $p<0.01$), CI ($t = 4.74$,$p<0.01$) and BI ($t = 4.7$, $p<0.01$).

In order to find out any difference in selecting the breeding habitats *of Aedes* mosquitoes in urban area, an entomological surveillance was undertaken in Manjery municipal area of Malappuram district. Of the total 1440 house premises searched, 381 were found to be positive for the presence of *Aedes* larvae (HI-26.45%) and of the total 2131 water holding containers/sources examined, 524 (CI-24.59%) were found positive for *Aedes* larvae. The Breteau index which relates the positive breeding sites and number of houses examined in urban area was 36.39.



	HI	CI	BI
Monsoon	39.8	28.2	57.9
Premonsoon	10.3	14.8	10

FIGURE 2. *Aedes* larval indices in monsoon and pre monsoon (Urban area)

The entomological surveillance in randomly selected urban area of Malappuram district showed that the mean score of HI in monsoon and pre monsoon were 39.8 ± 3.5 and 10.3 ± 3.4 respectively. The container index (CI) in monsoon and pre-monsoon were 28.2 ± 2.1 and 14.8 ± 1.5 respectively. The Breteau index (BI) in monsoon was 57.9 ± 10.2 whereas that of pre monsoon was 10.00 ± 1.8 (Fig. 2). An analysis was done to compare the *Aedes* larval indices during monsoon and pre monsoon. The mean values are tested by kolmogorvo-smirnov test and the result showed that the variances are equal for all the three indices ($p>0.05$). The mean scores in monsoon are significantly higher than that in pre monsoon at 1% level significance for the indices HI ($t = 5.82$, $p<0.01$), CI ($t = 4.72$, $p<0.01$) and BI ($t = 3.88$,$p<0.01$)

Statistical analysis was done to compare the *Aedes* larval indices in rural and urban areas in each season. The mean HI in rural and urban areas during monsoon were 33.1 ± 2.9 and 39.8 ± 3.5 respectively. Similarly the mean CI in rural and urban were 26.3 ± 1.5 and 28.2 ± 2.1 respectively. The mean score of BI in rural was 47.6 ± 6.9 whereas that of urban was 57.9 ± 10.2. The data were analysed using Kolmogorov-smirnov test and the result showed that the variances were equal for all the three indices ($p>0.05$). The t-test showed that there is no significant difference in mean scores between rural and urban areas in the monsoon months for the indices Hi ($t = 1.46$, $p>0.05$), CI ($t = 0.72$, $p>0.05$) and Bi ($T + 0.83$, $p>0.05$). A similar analysis was also done to compare the three indices in rural and urban area in the pre monsoon months. The mean HI in rural and urban areas during pre monsoonwas 8.0 ± 1.9 and 10.3 ± 3.4 respectively. The respective values for CI were 16.4 ± 1.3 and 14.8 ± 1.5. The Mean BI in rural was 8.3 ± 1.2 and urban was 10.00 ± 1.8. The kolmogorvo-smirnovtest showed that the variances are equal for all the three indices (p.0.05). The t-test showed that there is no significant difference in mean scores between rural and urban in pre monsoon months for the indices HI ($t = 0.60$, $p >0.05$), CI ($t = 0.81$, $p>0.05$) and BI ($t = 0.77$, $p >0.05$).

The distribution and eco-position of containers/ habitats may influence the site selection of *Aedes* mosquitoes for oviposition. The Breeding Preference Ratio (BPR) was calculated in order to find out the most preferred habitat selection of *Aedes* mosquitoes (Table 1). It has been noted that in rural area the BPR with respect to *Aedes* mosquitoes was more in coconut shells (1.26) followed by metal containers (1.25), plastic (1.23) and mud containers (1.06). The habitat preference of Dengue /chikungunya vectors in urban situation in the present study showed that the BPR was more in metal containers (1.34) followed by plastic (1.24), mud containers (1.22) and coconut shells (1.19).

The containers seen scattered in the peridomestic area of the study areas are broadly classified as man-made/artificial and natural. An analysis was done to find out the breeding preference of *Aedes* mosquitoes towards artificial or natural habitats in rural and urban set up. The mean BPR in rural and urban areas were 0.871 $\pm$ 0.1 and 0.872 $\pm$ 0.1 respectively. The kolmogorvo-smirnov test showed that the variances are equal for all the three indices ($p>0.05$). The t-test showed that there is no significant difference in the mean scores of artificial containers in rural and urban areas ($t = 0.07, p>0.05$). The BPR of natural containers in rural was 0.878 $\pm$ 0.1 and that of urban was 0.435 $\pm$ 0.1. The t-test showed that the natural habitat score in rural is significantly higher than that of urban ($t = 2.56$, $p<0.05$).

Landing collection of *Ae. albopictus* mosquitoes

The No./Man Hour density of female *Ae. albopictus* landing on human bait was recorded. The Per Man Hour (PMH) density of female *Ae.albopictus* in rural area during the monsoon and pre-monsoon months were 5.6 and 1.0 respectively. In urban areas the PMH density of *Ae.albopictus* in monsoon and pre-monsoon were 6.66 and 1.2 respectively.

DISCUSSION

Developmental activities, especially urban development associated with rapid growth of townships, have accentuated the problem of Vector-borne diseases, especially dengue, which is basically an urban disease (Dutta and Mahantha, 2006). With regard to dengue/chikungunya vector proliferation, human ecology is responsible for the creation of a mosquitogenic environment. In the present study, it has been found that *Ae.albopictus* was the predominant species prevalent in both rural and urban areas of Malappuram district. Among all the habitats examined in Anakkayam and Thrikalangode panchayat (rural) areas, the most preferred containers were coconut shells (BPR-1.26) reflecting the typical rural set up of the area. Containers are probably the most important factor determining the breeding of *Aedes* mosquitoes, since man-made containers are the major larval habitats in and near human habitation (Lee, 1991).

The *Aedes* survey carried out in Manjeri municipal area (urban) showed that most preferred breeding sites of *Aedes* mosquitoes were metal and plastic containers,

TABLE 1. Breeding habitats of *Aedes albopictus* in Rural and Urban areas of Malappuram District

Sl. No	Type of Breeding Habitats	Rural			Urban		
		Examined	Positive For *Aedes* larvae	Breeding preference Ratio (BPR)	Examined	Positive for *Aedes* larvae	Breeding preference Ratio (BPR)
Artificial							
1	Mudpots/Mud jars/Flower pots	236(13.09)	69(13.09)	1.06	303(14.22)	90(17.34)	1.22
2	Metal containers	270(14.98)	93(18.75)	1.25	325(15.25)	106(20.42)	1.34
3	Plastic containers	237(13.15)	80(16.13)	1.23	268(12.58)	81(15.61)	1.24
4	Glass bottles	189(10.49)	51(10.28)	0.98	234(10.98)	50(9.63)	0.88
5	Cement tank/cisterns	124(6.88)	22(4.44)	0.65	126(5.91)	20(3.85)	0.65
6	Grinding stones	112(6.22)	20(4.03)	0.65	133(6.24)	23(4.43)	0.71
7	Roof gutters	82(4.55)	16(3.23)	0.71	87(4.08)	10(1.93)	0.47
8	Over head tanks	85(4.72)	07(1.41)	0.3	115(5.40)	08(1.54)	0.29
9	Tyres	108(5.99)	30(6.05)	1.01	133(6.24)	34(6.55)	
Natural							
10	coconut shells	248(13.76)	86(17.34)	1.26	286(13.42)	83(15.99)	1.19
11	Tree holes	44(2.44)	08(1.61)	0.66	36(1.69)	03(0.19)	0.11
12	Stumps	17(0.94)	04(0.81)	0.86	14(0.66)	02(0.39)	0.59
13	Leaf axils	50(2.77)	10(2.02)	0.73	71(3.33)	09(1.73)	0.52

The figures in parentheses indicate percentage values

This probably reflects the attitude of urban people. Having increasingly prone to the consumerist culture, the people have fallen victim to the thrown away habit. House premises, market places, public places all are laden with scatterings and heaps of wastes mostly of non-degradable materials such as plastic, wrappers and carriers. These are capable of retaining water for many weeks especially during rainy season and pave for profuse breeding of *Aedes* mosquitoes (Rajendran, 2006). It has been noted that *Ae. albopictus* exhibited a wide spectrum of breeding habitats with a predilection for coconut shells in rural areas of Malappuram district. While describing the epidemiological factors favouring the outbreak of dengue in Kerala, Tyagi and Dash (2004) reported coconut shells/plastic cups are the most preferred breeding habitats of *Ae. albopictus*. While studying the breeding patterns of *Aedes stegomyia albopictus* in the peri urban areas of Calicut, Rao and George (2010) reported coconut shells and plastics are most favoured breeding sites of the vector mosquitoes.

Kerala is facing outbreak of DF/DHF since 1997 and chikungunya from 2006. *Ae.albopictus* is the most common vector species and has been incriminated as the sole vector of DF/DHF so far in Kerala (Das *et al*., 2004). This species has been reported in Kerala since 1915 (Barraud, 1931). It is also reported breeding in latex cups in rubber plantations (Sumodhan, 2003), plastic cups (Hiriyan *et al* , 2003), rodent damaged cocoa pods in cocoa plantations (Hiriyan and Tyagi, 2004), several man-made and natural habitats (Rajendran *et al*., 2006; Jomon *et al*.,2009; Anish *et al*., 2011; Bhaskar Roa, 2010), leaf sheaths of Areca palms (Regu *et al*.,2008) and leaf axils of many plants (Eapen *et al*., 2010). In an entomological surveillance carried out in Alappuzha District Sheela Devi (2011) observed that the most preferred breeding sources based on Breeding Preference Ratio (BPR) was in tyres in both rural and urban areas. *Ae. albopictus* mosquitoes prefer to breed in man-made/ artificial and natural habitats. While analyzing the breeding preference it has been found in the present study that there was no significant difference in the mean score of artificial containers both in rural and urban set up. It is possible because of rapid urbanization taking place in Kerala. In most of the districts of Kerala the demarcation between rural and urban is insignificant and this stands true for Malappuram district too. However it has been found in the present study that the natural habitat score in rural areas of Malappuram district was significantly higher than that of urban, indicating the availability of rich plant sources for *Aedes* mosquitoes to breed and flourish.

In the present study it has been found that the *Aedes* prevalent indices are high during monsoon in rural and urban areas of Malappuram district. Generally a HI greater than 5% and BI greater than 20 for any locality is dengue sensitive. The high *Aedes* indices noted in both rural and urban areas indicate impending outbreak of DF/chikungunya in these areas. With respect to human landing catches of *Aedes* adult females, areas with densities greater than 2 Man Hour are considered high risk where as those less than 0.2 are low risk. In the present observation, the Per Man Hour female *Aedes* density in rural and urban areas during monsoon was 5.6 and 6.6 respectively indicating high risk for focal outbreak of dengue fever and chikungunya in these

areas. 1886 suspected and 16 serologically confirmed chikungunya cases reported from Malappuram district during 2007 substantiate the veracity of this observation.

Dengue and chikungunya are in many ways the prototype of 'Man-made diseases'. The larval habitats of *Aedes* vector mosquitoes are artificial containers found in and around the houses in most settings where transmission is occurring. Promotion of elimination or control of larval habitats at the household level through partnerships between community groups, local bodies and the private sector is one of the cornerstones of a successful integrated strategy for preventing DF/DHF and chikungunya (Gubler and Clark,1994). Control of larval containersin habitats is one of the several sets of measures that need to be promoted, but it is still to receivesufficient attention and effort. Integrated and effective vector control strategies depending on the terrain and local conditions are to be implemented with the active participation of the community. It is only through gaining conscientized awareness of people and ensuring participatory involvement of the society, effortsto control these dreaded diseases will bear fruit.

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Molecular characterization of *Culex quinquefasciatus* Say (Diptera: Culicidae), a filarial vector of Bankura district, West Bengal, India

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ABSTRACT: We examined the molecular characters of *Cx. quinquefasciatus* collected from different filarial endemic regions of Bankura district of West Bengal, India, using 28S ribosomal RNA gene nucleotide sequence. The mosquitoes were examined for *Wuchereria bancrofti* infection. Per-man hour density (PMD) of the vector was 46.86. The infection and infectivity rates of *Wuchereria bancrofti* in vector population were 4.83% and 0.97% respectively. Analysis of 28S rRNA gene sequence of *Cx. quinquefasciatus* revealed that AT content and GC content were 36.11% and 63.89% respectively. Phylogenetic tree was prepared using the Neighbor-Joining (NJ) method. Phylogenetic tree revealed that *Cx. quinquefasciatus* isolate SNC 25 branched with *Cx. quinquefasciatus* isolate 09KAC(HM802159) showing maximum identity (96%) with 09KAC(HM802159). HM807289, 02KAN(HM807287), 08CHE(HM802158), 10TRI(HM802160), 03TNV(HM807288), 06VIL(HM802156), 01VEL(HM802153), 04VNR(HM802154); 95% identity with 05MDU(HM802155) and CpiLDH09 (DQ401446) and 93% identity to 07SAL(HM802157).

KEYWORDS: *Culex quinquefasciatus*, filarial vector, 28S rRNA sequence, phylogenetic tree

INTRODUCTION

Culex, Aedes, Mansonia and *Armigeres* transmit the most important human diseases like malaria, filarial and some arboviruses etc. (Porter *et al.*, 1993). As important vectors of filaraia. *Culex quinquafasciatus* and *Aedes spp* have affected more than 90 million human being worldwide (Porter *et al.*, 1993). In India, about 27 million

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people have been reported to be microfilarial carriers and about 20 million people are diseased and the states like Andhra Pradesh, Bihar, Jharkhand, Gujarat, Kerala, Maharastra, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal are endemic for filariasis (National Filaria Control Programme, Annual Report 2003). In West Bengal, Bankura is a highly endemic for filariasis. The report on filarial situation in West Bengal is very limited (Das *et al.*, 2003). From West Bengal, it has been reported that about 0.95 and 0.03 million peoples are carriers and diseased, respectively (National Filaria Control Programme, Annual Report 2003). *Cx. quinquefascitus* has been incriminated as a filarial vector in some parts of West Bengal (Rudra and Chandra, 2000; Chandra *et al.*, 2007). It is widely distributed in different parts of India (Rahuman *et al.*, 2008). Present piece of work has been designed to determine the molecular characterization of the filarial vector *Cx. quinquefasciatus* Say captured from filaria-endemic areas of Bankura district of West Bengal, India.

MATERIALS AND METHODS

Mosquito collection was done following the method of WHO (1972). *Cx. quinquefasciatus* mosquitoes were captured from selected eight spots of Bankura district of West Bengal, India. Mosquitoes were dissected and examined for *Wuchereria bancrofti* infection. After isolation of DNA, PCR amplification of *Cx. quinquefasciatus* DNA was done. Using 28s rRNA primers, the ~400 bp fragment was amplified using Taq DNA Polymerase. The PCR product was purified and sequenced using both forward and reverse primers and aligned. The respective region was amplified using 28s rRNA forward and reverse primers. The sequence was submitted NCBI Gen Bank and assigned the GI number as HM450314.The mole-percentage of Adenine, Thymine, Guanine and Cytosine were determined. Fingerprint of nucleotide was done following Lo and Golding (2007) and restriction map was prepared following Vincze *et al.* (2003). To understand the most similar available sequences, a BLAST search was done in NCBI databases and closest rRNA gene sequences of *Cx. quinquefasciatus* were retrieved from NCBI database, where as *Aedes aegypti* isolate A20 (DQ397937) was taken as an out-group. Evolutionary distances were calculated using the method of Jukes and Cantor (Jukes and Cantor, 1969) and a Phylogenetic tree was prepared by 'neighbor-joining' method (Saitou and Nei, 1987; Tamura *et al.*, 2004).

RESULTS AND DISCUSSION

Vector infection and infectivity rates in Bankura of West Bengal, India were 4.83% and 0.97% respectively. Per-man hour density (PMD) was recorded to be 46.86. Higher prevalence of indoor-resting *Cx. quinquefasciatus* was seen in rainy season because of water logging in many new sites besides the perennial drainage sources. Infection and infectivity rates were also higher in rainy season. The temperature range (25° to 32°C) and humidity range (75% to 95%) were found favorable for the vector and parasites. Insect-borne diseases are now being the major causes of illness and death (Pavela, 2009). Lymphatic filarisis is a major health problem in India (Agrawal *et al.*,

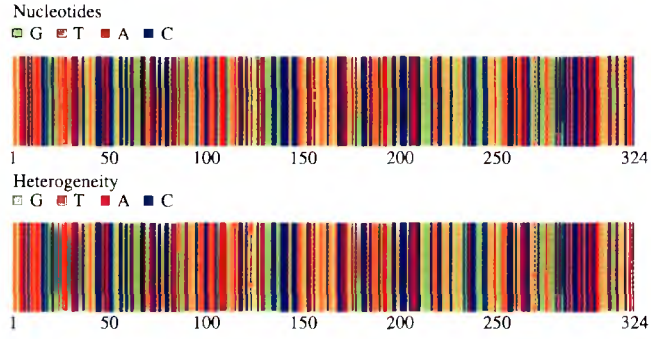

FIGURE 1. Fingerprint of nucleotides of 28S rRNA gene sequence of *Culex quinquefasciatus* isolate SNC25 (HM450314).

TABLE 1. Alignment view and distance matrix table taking *Culex quinquefasciatus* isolate SNC 25 (HM450314) 28S ribosomal RNA gene sequence as reference sequence

Organisms	NCBI accession number	Length of DNA	Maximum identity	*E*-value
Culex quinquefasciatus	HM807289	501 bp	96%	3e−149
Culex quinquefasciatus isolate 02 KAN	HM807287	500 bp	96%	3e−149
Culex quinquefasciatus isolate 10 TRI	HM802160	493 bp	96%	3e−149
Culex quinquefasciatus isolate 09 KAC	HM802159	488 bp	96%	3e−149
Culex quinquefasciatus isolate 08 CHE	HM802158	490 bp	96%	3e−149
Culex quinquefasciatus isolate 06 VIL	HM802156	488 bp	96%	3e−149
Culex quinquefasciatus isolate 04 VNR	HM802154	489 bp	96%	3e−149
Culex quinquefasciatus isolate 01 VEL	HM802153	488 bp	96%	3e−149
Culex quinquefasciatus isolate 03 TNV	HM807288	495 bp	96%	1e−147
Culex quinquefasciatus isolate 05 MDU	HM802155	491 bp	95%	3e−144
Culex quinquefasciatus isolate 07 SAL	HM802157	486 bp	93%	2e−126
Culex pipiens pipines clone CpiLDH09	DQ401446	291 bp	95%	4e−113

2006). *Cx. quinquefasciatus* is the principal vector of Bancroftian filariasis (Cook and Zumla, 2003). As filariasis is not fatal, it is a less attended disease and limited attention has been paid to contain the disease (WHO, 1992). Through limited surveys, it has been estimated that the annual economic loss due to filariasis is about Rs. 5000/- crores in India Ramaiah and Vijay Kumar (2000) besides the social stigma attached to it. To make a useful control strategy it is imperative to know the genetic diversity of the filarial vectors. Genetic diversity of the vectors influences the progression of parasites and transmission of disease. Exploitation of genetic variability among vectors could pave the path for the alternative strategies for vector management (Sharma *et al.*, 2010). Miller *et al.* (1996) examined Ribosomal DNA sequence divergence in

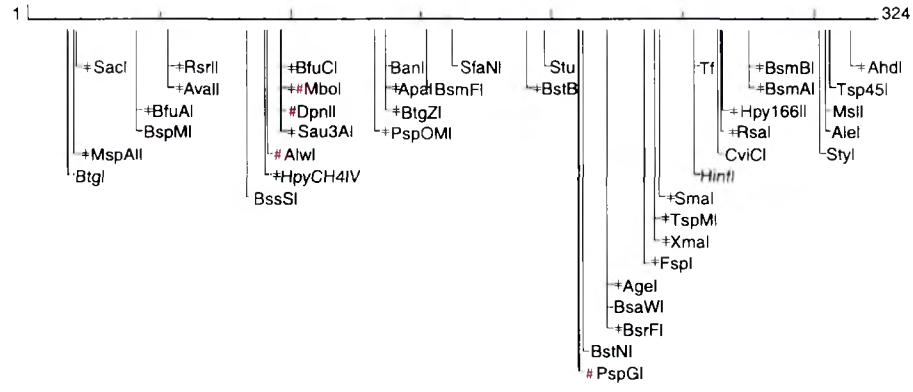

FIGURE 2. Restriction map of 28s rRNA gene sequence of *Culex quinquefasciatus* isolate SNC25 (HM450314).

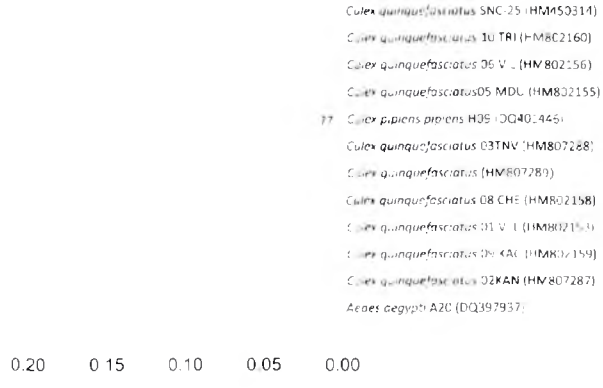

FIGURE 3. Neighbour-joining Tree of *Culex quinquefasciatus* isolate SNC25 (HM450314).

the internal transcribed spacer regions (ITS-1 and ITS-2) for fourteen species and four subgenera (sixty-two clones) in the mosquito genus *Culex* (Diptera: Culicidae) and showed that each of the four subgenera was monophyletic at confidence probabilities of 70–99%. *Culex* (*Lutzia*) formed the sister group of *Cx.* (*Culex*). Analysis of 28S rRNA gene sequence of *Cx. quinquefasciatus* isolate SNC25 (HM450314) showed that the mole-percentage of AT and GC content were 36.11% and 63.89% respectively. The fingerprint and restriction map of 28S rRNA gene sequence of *Cx. quinquefasciatus* isolate SNC25 (HM450314) have been shown in Figs. 1 and 2 respectively. Phylogenetic tree along with branch lengths has been shown in Fig. 3. Taking *Cx. quinquefasciatus* isolate SNC 25 (HM450314) 28SrRNA gene sequence as reference sequence, the alignment view and distance matrix table was prepared and displayed in Table 1. Alignment view table (Table 1) and phylogenetic tree (Fig. 3) showed that *Cx. quinquefasciatus* isolate SNC 25 branched with the other *Cx. quinquefas-*

ciatus isolates with 77% bootstrap support having maximum identity (96%) with *Cx. quinquefasciatus* isolates 09KAC(HM802159), HM807289, 02KAN(HM807287), 08CHE(HM802158), 10TRI(HM802160), 03TNV(HM807288), 06VIL(HM802156), 01VEL(HM802153), 04VNR(HM802154); 95% identity with the isolates *Cx. quinquefasciatus* 05MDU(HM802155) and *Cx. quinquefasciatus* CpiLDH09(DQ401446) but 93% identity to the isolate *Cx. quinquefasciatus* 07SAL(HM802157).

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Management of banana rust thrips, *Chaetanaphothrips signipennis*

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ABSTRACT: Field experiment conducted during 2008–09 and 2009–10 to manage banana rust thrips *C. signipennis* showed the lowest thrips infestation was recorded by bud injection treatment of Imidacloprid (16.88%) followed by chlorpyriphos (24.94%) which was *at par* with bud injection of azadirachtin (25.74%). Imidacloprid bud injection also resulted in highest yield (57.78 t/ha) and bunch weight (18.72 kg) even though it was *at par* with chlorpyriphos (17.90 kg and 55.24 t/ha) and neem oil (17.87 kg and 55.15 t/ha). The treatment comprised bunch sleeving of fruit bunches recorded highest infestation of fruit bunch (54.63%) and lowest recorded bunch weight (15.55 kg) and yield (47.97 t/ha).

KEYWORDS: Banana rust thrips, *Chaetanaphothrips signipennis*, management.

Banana (*Musa parasidica*) is one of the most important fruit crop grown all over the world. It is also one of the most important fruit crop grown in India and particularly in the south Gujarat especially in the districts viz., Surat, Narmada, Tapi, Bharuch and Navsari. Simmonds (1966) has given an annotated list of 182 pests of banana from all over the world. Among them corm weevil, pseudostem weevil, aphid, lace wing bug, leaf eating caterpillars, hairy caterpillar and thrips are very important. Thrips complex of banana is very important so far the quality of the fruit is concerned. The thrips complex of banana includes the flower thrips, leaf thrips and rust thrips etc. Out of these species, rust thrips (*Chaetanaphothrips signipennis*) is very important one because this is directly responsible for the qualitative losses in banana. The primary host of *C. signipennis*, are banana, anthurium and bracaena although not specific to bananas (*Musa spp.*) affects banana as its main host. Its feeding has also been recorded on citrus and tomato and a number of weed species (Simmonds, 1966). The banana thrips, *C. signipennis*, is of economic importance in many banana-producing countries. Circular rusty-red patches appear on affected fruits and later there

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TABLE 1.

T_1	Bunch sleeving at shooting stage (100 gauge thickness, 60% ventilation)
T_2	Spray of azadirachtin 1% EC at 5 ml/liter of water
T_3	Neem seed kernel extract at 2 ml/liter of water
T_4	Bud injection of azadirachtin (1% EC) at 5 ml/liter of water (2 ml/bud)
T_5	Spray of neem oil (0.5%) at 2 ml/liter of water
T_6	Spray of pongamia oil at 2 ml/liter of water
T_7	Bud injection of imidacloprid at 0.1 ml/500 ml of water (1 ml/bud)
T_8	Spray of chlorpyriphos 0.05% (2.5 ml/liter) (Standard check)
T_9	Control (Unsprayed)

is superficial cracking of the skin or sometimes splitting of the fruit. The affected banana fruits become reddish and rusty in appearance and they will be not useful in export. Considering the importance of the banana as one of the most important fruit of south Gujarat and have a great export potential, the present study was undertaken to manage this very important pest of banana.

The present investigations were carried out at Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat during 2008–09 and 2009–10 on banana cv Grand Naine. Total nine treatments including those in control with randomized block design (RBD) were made and each treatment was replicated three times. The first spray was imposed at the time of shooting stage and the second spray after opening of all hands. The treatment comprisesd bud injection at the time of initiation of flower bud (upright position). Observation on rust infestation on figures at harvest and bunch weight was recorded to determine the effectiveness of the treatments. The treatment details are as depicted in Table 1.

The efficacy of various treatments against rust thrips of banana *C. signipennis* was presented in the Table 1. The data revealed that all the treatments were found superior when they were compared to the untreated control. There was no significant difference in the total fruits per bunch in different treatments. The maximum number of thrips infested fruits was recorded in the treatment control (105.23) which was at par with the treatment comprises with bunch sleeving at shooting stage (100.00). The minimum infested fruits per bunch were recorded from the treatment comprises of bud injection of imidacloprid (T_7) i.e. 13.00 fruits per bunch and it was followed by treatments T_4 (28.50 fruits/bunch) and T_8 (27.25 fruits/bunch). In terms of per cent infestation, the highest number of thrips infested fruits was recorded in T_9 (58.24%) and it was at par with T_1 (54.63%). The minimum per cent fruit infestation was recorded in T_7 (16.88%) and it was followed by T_5 (30.41%) and T_8 (24.94%). The highest bunch weight was recorded in the treatment comprises of bud injection of imidacloprid (T_7) i.e. 18.72 kg/plant which was at par with other treatments viz., T_2, T_3, T_4, T_5 and T_6. The lowest bunch weight was recorded in case of untreated control (13.99 kg/plant) and it was at par with T_1 (15.55 kg/plant). Similar trends were followed when the yield data were calculated on the hectare basis. The highest yield was also recorded in the treatment T_7 (57.78 t/ha) and it was minimum in the treatment T_9 (43.19 t/ha). So,

TABLE 2. Efficacy of different treatments on banana rust thrips, *Chaetanaphothrips signipennis* (pooled of two years)

Treatments	Total fruits per bunch	No. of infested fruits per bunch	% infestation of fruits/bunch	Bunch weight (kg/ha)	Yield (t/ha)
T_1	125.50	100.00^{a}	54.63^{a}	15.55^{bc}	47.98^{bc}
T_2	150.25	36.75^{bc}	29.69^{bc}	17.32^{ab}	53.46^{ab}
T_3	150.50	36.00^{bc}	29.28^{bc}	17.35^{ab}	53.53^{ab}
T_4	150.75	28.50^{cd}	25.74^{cd}	17.37^{ab}	53.61^{ab}
T_5	152.75	39.00^{b}	30.41^{b}	17.87^{a}	55.15^{a}
T_6	149.00	39.50^{b}	31.06^{b}	17.43^{ab}	53.79^{ab}
T_7	153.25	13.00^{e}	16.88^{e}	18.72^{a}	57.78^{a}
T_8	150.25	27.25^{d}	24.94^{d}	17.90^{c}	55.24^{a}
T_9	149.00	107.25^{a}	58.24^{a}	13.99^{c}	43.19^{c}
CD at 5%	NS	8.70	4.12	1.94	6.00

from this study it can be concluded that imidacloprid and chlorpyriphos recorded lower bunch infestation and higher bunch weight and yield. the biocides like azadirachtin provided nearly the same results for the management of the rust thrips in banana. Pinese (1984) reported that injection of imidacloprid or any other chemical pesticide for scab moth provides early bunch protection against rust thrips. Further, extended protection up to harvest should then be provided by one insecticide application to the bunch. Pena *et al.* (2006) also reported that imidacloprid, acetmiprid, fenpropathrin, milbemectin, zeta cypermethrin and chlorpyriphos were very effective in controlling the flower thrips in mango. Under the present study imidacloprid also showed its superiority over rest of the treatments and very effective in reducing the qualitative losses in banana hence confirm the earlier findings.

ACKNOWLEDGEMENT

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Reporting of two new species of genus *Thanatarctia* (Lepidoptera : Arctiidae: Arctiinae) from India

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ABSTRACT: Two new species i.e., *costalis* and *collarlis* of genus *Thanatarctia* Butler have been described from India. Both the species are closely allied to *infernalis* Butler, the type species of this genus, as far as general coloration, maculation and wing pattern are concerned. These species can be distinguished from each other on the basis of distinct genitalic structures and other morphological characters. A key to the known Indian species of this genus has been given.

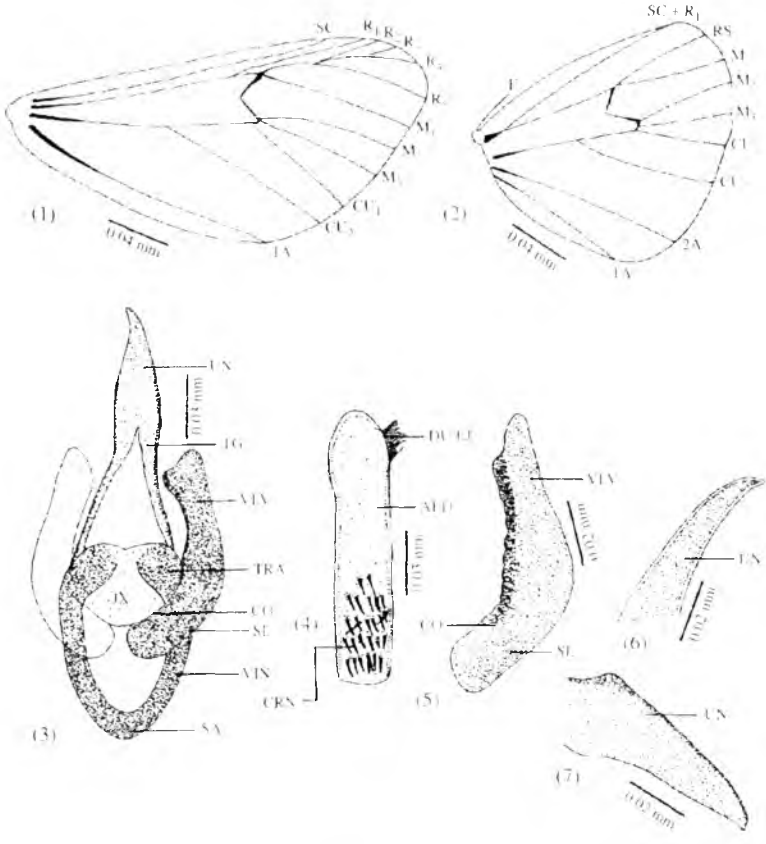
KEYWORDS: Arctiidae, *Thanatarctia*, *costalis*, *collarlis*, new species.

INTRODUCTION

During surveys undertaken for the collection of tiger moths in North-Eastern States, a complex phena of ten specimens were collected from Jatinga, Assam. All the individuals possessed a similar type of coloration, maculation and wing pattern and their tentative sorting has led to the inference that these individuals belong to a single species i.e., *infernalis* Butler. But after critical examination of morphological characters, particularly the genitalic features, it has been concluded that these individuals belong to three distinct species. One species has been identified as *Thanatarctia infernalis* Butler with the help of relevant literature (Hampson, 1894, 1901; Koda, 1988; Watson *et al.*, 1980) and comparisons with the identified collections of National/International museums. The remaining two species are described and illustrated here as new to science. The terminology for genitalia has been followed after Klots (1970).

Thanatarctia costalis n. sp.

Head with vertex and frons covered with crimson scales. Antenna with scape studded with crimson scales; flagellum black, branches laiden with brown scales. Eyes black. Labial palpus porrect, just reaching lower level of frons; all segments covered with black scales but on inner margins decorated with crimson scales.



FIGURES 1–7. *Thanatarctia costalis* n. sp. 1. Forewing; 2. Hindwing; 3. Male genitalia; 4. Aedeagus; 5. Valva – ventral view; 6. & 7. Uncus – laternal view.

Thorax and tegula clothed with brown black scales, latter edged with crimson scales; collar crimson, with dark margins; pectus crimson. Forewing with ground colour brown black; a basal crimson spot; subbasal narrow pale crimson streaks on both margins of cell and in cell, one in submedian area; an antemedial streak in submedian fold; veins streaked with brown and suffused with some crimson scales; fringe black; underside with a broad crimson streak in cell; R_2 – R_5 stalked from upper angle of cell; Cu_2 from beyond middle of cell. Hindwing with ground colour brown black; a basal patch of crimson scales; a crimson medial spot on costa; a medial spot prominent on underside; R_5 and M_1 shortly stalked from upper angle of cell; Cu_2 from two-thirds of cell. Legs with coxae furnished with crimson scales, spotted with black; femora black above, underside pale yellow, with slight crimson tinge, fore and mid tibia yellow, streaked with black; hind tibia with distal portion black; tarsi black; outer tibial spurs almost of equal size of inner ones.

Abdomen decorated with crimson scales, with a dorsal black maculate line; lateral and sub lateral series of black spots.

Male genitalia

Uncus small, triangular well sclerotized, broad at base, narrowing towards tip; tegumen with both of its walls equally wide, longer than vinculum; vinculum V-shaped; saccus and costa narrow; cucullus and valvula merged, a triangular projection along outer margin towards tip; juxta large trapezoid, theca depressed in middle; transtilla long. Aedeagus short, tip rounded; vesica armed with numerous spine-like cornuti.

Female genitalia

Not examined.

Wing Expanse (Half) : Male: 15 mm

Material Examined

Holotype : Assam: North Cachar Hills, Jatinga, 29. IX. 1995, 1 ♂.
Paratype : Assam: North Cachar Hills, Jatinga, 26. IX. 1995, 1 ♂.

Remarks

The present species is closely allied to the type species. *Thanatarctia infernalis* Butler with respect to ornamentation of head, thorax and abdomen; structure of labial palpus and wing venation. However, it differs from *infernalis* in having a basal crimson spot, subbasal crimson streaks on both margins of cell and an antemedial streak on submedian nervure on forewing. Other important differences are the following: hindwing with crimson medial spot on costa; male genitalia with vinculum U shaped different valva, aedeagus without any sclerotization at distal end.

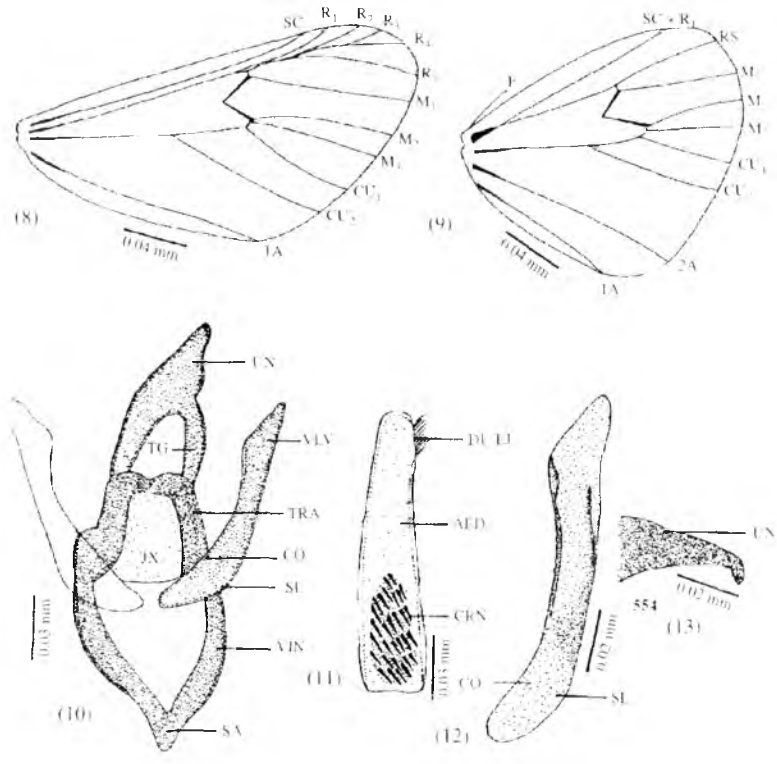
Etymology

The name of the species pertains to costa of forewing with a crimson spot.

Thanatarctia collarlis n.sp.

Head with vertex and frons furnished with rose-red scales, latter with black scales on sides. Antenna with scape laiden with rose-red scales; flagellum black. Eyes black with brown lining. Labial palpus short and porrect, not reaching lower level of frons; all segments decorated with black scales, inner margins studded with crimson scales.

Thorax clothed with black-brown scales; collar crimson, margins darker, bearing black spots; tegula black, edged with pale crimson scales; pectus crimson. Forewing with ground colour black-brown; a basal patch of pale crimson scales; veins with brown scales, slightly tinged with crimson; a crimson tinged line on submedian nervure; fringe black; veins $R_2 - R_5$ stalked from before upper angle of cell; M_2 and



FIGURES 8–13. *Thanatarctia collarlis* n.sp. 8. Forewing; 9. Hindwing; 10. Male genitalia; 11. Aedeagus; 12. Valva – ventral view; 13. Uncus – laternal view.

M_3 from lower angle of cell; Cu_2 from two-thirds of cell. Hindwing with ground colour brown black; a basal patch of crimson scales; fringe black; vein Rs originating from before upper angle of cell; M_2 from just above lower angle of cell; Cu_2 from beyond two-thirds of cell. Legs with coxae dressed with crimson scales; femora black above, underside with rough yellowish and crimson tinged scales; tibia and tarsi black, streaked with pale crimson tinged yellow scales; tibial spurs almost of equal length.

Abdomen covered with crimson scales; with dorsal black line; lateral and sublateral series of black spots.

Male genitalia

Uncus short, triangular swollen at base, gradually narrowing towards distal end, tip curved and sclerotized; tegumen strongly sclerotized, as long as vinculum; vinculum broad V-shaped; saccus narrow and pointed. Valva long and narrow; costa and sacculus narrow, distal end slightly broad, rounded, cucullus and valvula fused, semi-sclerotized, a slight triangular projection at distal end; juxta large, shield-like; transtilla

long, well developed. Aedeagus broad at base, tip rounded and narrow; vesica armed with a bunch of well arranged long spines.

Female genitalia

Not examined.

Wing Expanse (Half) : Male: 16 mm

Material Examined

Holotype: Assam: North Cachar Hills, Jatinga, 28.IX.1995, 1 ♂.

Paratype: Assam: North Cachar Hills, Jatinga, 28.IX.1995, 1 ♂; 30.IX.1995, 1 ♂.

Remarks

The species under reference is very much near to *infernalis* Butler and also allied to another new species i.e., *costalis*. But, this species is distinct in several features such as presence of a black spot on collar; a crimson tinged streak on submedian nervure and vein M_2 arising from angle of cell in forewing. Male genitalia is also distinct uncus being small with curved tip; valva appearing narrow and for the absence of rounded sclerotized projection on aedeagus.

Etymology

The name of the species is based on the collar, having black spots.

***Thanatarctia infernalis* Butler**

Thanatarctia infernalis, Butler, 1877, A.M.N.H. (4) 20: 395; id, 1879, Ill. Het. B.M. 3: 7; Hmpsn., 1901, Cat. Lep. Phal. 3: 312, Kirby, 1892, Cat. Het. 1: 277; Koda, 1988, Tyo to Ga, 39(1): 11-19.

Wing Expanse (Half) : Male: 16 mm

Material Examined

Assam: North Cachar Hills, Jatinga, 25.IX.1995, 1 ♂; 02.IX.1995, 1 ♂; 30.IX.1995; 1 ♂; 01.X.1995, 2 ♂ ♂.

Distribution: India: Assam; Elsewhere: China, Japan.

Remarks

The male genitalia of this species has already been studied and illustrated by Koda (1988). Hence, the description of the species has not been included in the present communication.

KEY TO THE KNOWN INDIAN SPECIES OF GENUS *THANATARCTIA* BUTLER

1. Head with vertex covered with rose red scales; labial palpus fringed with crimson scales on inner side; wings with ground colour black brown 2
   Head thorax covered with pale buff or ochreous scales; labial palpus not fringed with crimson scales on inner side; wings with ground colour pale buff or ochreous 4
2. Forewing with veins R_2 to R_5 stalked before upper angle of cell; hindwing with no crimson spot on costa; male genitalia with vinculum V-shaped; valva narrow below middle 3
   Forewing with veins R_2 to R_5 stalked before upper angle of cell; hindwing with crimson spot on costa; male genitalia with vinculum U-shaped; valva broad below middle ..... *costalis* n.sp.
3. Collar spotted with black; forewing with vein M_2 arising from lower angle of cell; hindwing with vein Rs from before upper angle; male genitalia with aedeagus of moderate size, without any sclerotization; uncus with tip curved ..... *collarlis* n. sp.
   Collar without any black spot; forewing with vein M_2 arising from just above lower angle of cell; hindwing with vein Rs from upper angle of cell; male genitalia with aedeagus rather long, with a rounded sclerotization or distal end bearing spines; uncus with tip not curved ..... *infernalis* Butler
4. Thorax with dorsal medial streak; legs with femora decorated with crimson scales on upper side; legs with femora decorated with crimson scales on upper side; abdomen with dorsal, lateral and sub lateral series of black spots; male genitalia with valva long; aedeagus without any sclerotization at distal and ..... *mona* (Swinhoe)
   Thorax without dorsal medial streak; legs decorated with yellow scales; abdomen with dorsal and lateral series of black spots; male genitalia with valva of moderate length; aedeagus with rounded sclerotization on distal end 5
5. Forewing with vein M_2 from lower angle of cell, M_1 to R_5 stalked from upper angle; male genitalia with vinculum V-shaped 6
   Forewing with M_2 from above angle; M_1 from upper angle, R_2–R_5 stalked from upper angle; male genitalia with vinculum U-shaped 7
6. Forewing with medial and post-medial series of fuscous spots; hindwing with veins M_2 and M_3 shortly stalked from lower angle, Rs and M_1 stalked from upper angle of cell; male genitalia with uncus of moderate size, valva long, was smooth; juxta saucer-shaped ..... *flavens* (Moore)
   Forewing without medial and post-medial series of fuscous spots; hindwing with vein M_2 well above angle & M_3 from lower angle of cell, Rs & M_1 from upper angle; male genitalia with uncus small, valva of moderate size, broad to base, walls corrugated ..... *crispens* Kaleka
7. Forewing with post-medial series of spots, running round end of cell; hindwing with veins Rs and M_1 from upper angle of cell; male genitalia with uncus short, with lateral margins corrugated; aedeagus of moderate size, walls wrinkled beyond middle ..... *kodai* Kaleka

Forewing without post-medial series of spots; hindwing with Rs and M_1 shortly stalked from upper angle of cell; male genitalia with uncus of moderate size, lateral margins smooth; aedeagus long, with anterior tip rounded ..... *saccuens* Kaleka

Abbreviations

1A – First anal vein; 2A – Second anal vein; ACC. SC – Accessory sac; AED – Aedeagus; ANT.APO – Anterior apophyses; CO – Costa; CRN – Cornuti; CRP. BU – Corpus bursae; CU – Cucullus; Cu_1 – First cubital vein; Cu_2 – Second cubital vein; DU.BU – Ductus bursae; DU.EJ – Ductus ejaculatoris; G.P – Genital plate; JX – Juxta; M_1 – First medial vein; M_2 – Second medial vein; M_3 – Third medial vein; PA. P – Papilla analis; PO. APO – Posterior apophyses; R_1 – First radial vein; R_2 – Second radial vein; R_3 – Third radial vein; R_4 – Fourth radial vein; R_5 – Fifth radial vein; RS – Radial sector; SA – Saccus; Sc – Subcosta; $Sc+R_1$ – Subcosta and radial vein; SIG – Signum; SL – Sacculus; TG – Tegumen; TRA – Transtilla; UN – Uncus; VES – Vesica; VIN – Vinculum; VLA – Valvula.

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The author is thankful to Dr. N. Koda for proper guidance and his valuable reprints. The financial assistance rendered by CSIR, New Delhi is gratefully acknowledged.

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Sexing of the home invading darkling beetle *Mesomorphus villiger* (Coleoptera: Tenebrionidae: Opatrini)

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ABSTRACT: *Mesomorphus villiger* (Blanchard, 1853) (Coleoptera: Tenebrionidae: Opatrini) is a litter dwelling home invading nuisance beetle in south India. Lack of data in externally visible characters makes sex determination of *M. villiger* for biology studies difficult. Sexing of *M. villiger* based on a previously unnoticed externally visible gender-specific character and the methodology to be employed for sexing live specimens are detailed out.

KEYWORDS: *Mesomorphus villiger*, tobacco ground beetle, *Luprops tristis*, sexing, sternal notch method

INTRODUCTION

Mesomorphus villiger (Blanchard, 1853) (Coleoptera: Tenebrionidae: Opatrini) (Fig. 1), known as 'the tobacco ground beetle' in tobacco growing belts in the Deccan region (Sitaramaiah *et al.*, 1999), is commonly present in the leaf litter of many trees especially in the rubber plantations in south India. Home invasion of huge aggregations of *M. villiger* (Fig. 2) into residential buildings, nocturnal movements and release of an irritating, odoriferous secretion that causes mild skin burns and morphological similarity lead to its misidentification as the nuisance pest *Luprops tristis* (Vinod *et al.*, 2008) in many regions. Biological studies and culturing of the species requires sex determination of adults based on externally visible characters which would not harm the specimens. However, non-record of externally visible characters makes their sexing difficult and squeezing out the genitalia is the only option for sexing the specimens for biological studies as in the case of other Tenebrionids (Crowson, 1981). In the present effort, a novel methodology for sexing *M. villiger* is described and survivability of the specimens following the methodology is tested.

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MATERIALS AND METHODS

Collection and identification

Mesomorphus villiger adults were collected from a residential building at Calicut by handpicking during May 2012.

Identification was done following Mathews *et al.* (2008) and Ferrer (2002) and confirmed by comparing with the verified specimens in the Coleoptera collections of the host institution.

Sexing of live specimens and culture of sexed specimens

Beetle was held by the left thumb and index finger, placed on a glass slide kept on the stage of a stereo zoom microscope (25 X magnification) with the ventral surface facing up and with the posterior part of the beetle away from the worker, while the right hand was used to operate a # 4 watch makers forceps with blunted tips. Care was taken to immobilize the hind legs, otherwise the beetle ruptured the defensive gland reservoirs with its legs and the oily defensive secretion made the slide unclear. When the apex of the last visible abdominal sternite (7th) was gently lifted with the forceps, edges of 8th sternite and last outer tergite (8th) became visible. Line drawings of dimorphic character on the 8th sternite were made using Camera Lucida. Body length was measured from the anterior margin of the pronotum to the apex of elytra (Merkl and Kompantzeva, 1996). All measurements are in millimeters.

Specimens were paired and kept separately in clay vessels half filled with soil, litter and covered with nylon net and fed with tender rubber (*Hevea braziliensis*) and then the extent of mortality was checked. A few cotton balls moistened with drops of distilled water was kept in each clay vessel.

RESULTS

Sex differentiation is based on the presence of a notch on the posterior margin of 8th sternite of males which is absent in the females (Fig. 3). It is applicable to other species of the genus *Mesomorphus* from south India (*M. kulzeri*, *M. gridelli* and *M. striolatus*). Other genera of tribe Opatrini from the region (*Gonocephalum* and *Sclerum*) do not possess the sternal notch and their sexing is not possible using this character. Other than *Luprops tristis* of the tribe Lupropini, Tenebrionid species belonging to nine tribes (Lupropini, Lagrini, Amarygmini, Alphitobini, Opatrini, Bolitophagini, Platynotini, Ulomini and Toxicini) recorded from south India does not have sternal notch and it makes their sexing impossible using the character. In all the tenebrionid species recorded from the Indian subcontinent, anterior edge of sternites 8 and 9 in males are without a median strut contrary to the record (Lawrence *et al.*, 1999), that some members of the subfamilies Lagriinae and Alleculinae of the family Tenebrionidae bears the median strut on the anterior edge of the 9th sternite.

All beetles sexed by the 'sternal notch method' survived. Verification of the method by observation of the male genitalia (aedeagus) proved that the method was correct.

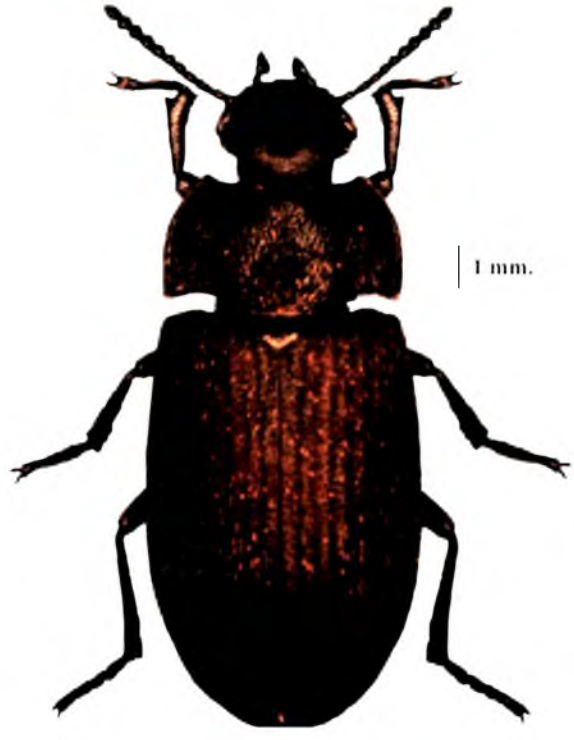

FIGURE 1. *Mesomorphus villiger* (Blanchard, 1853) Habitus.

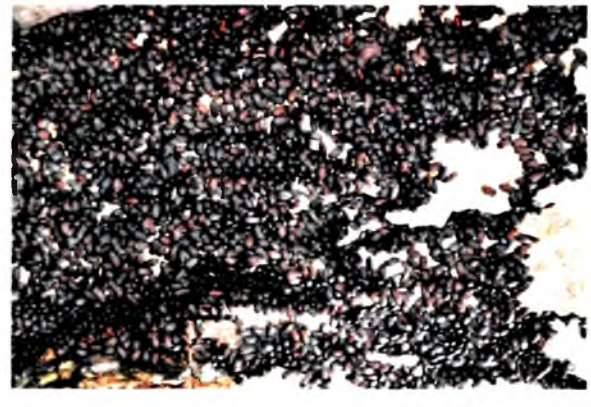

FIGURE 2. *Mesomorphus villiger* aggregations on the bark of *Samanea saman* (Rain tree).

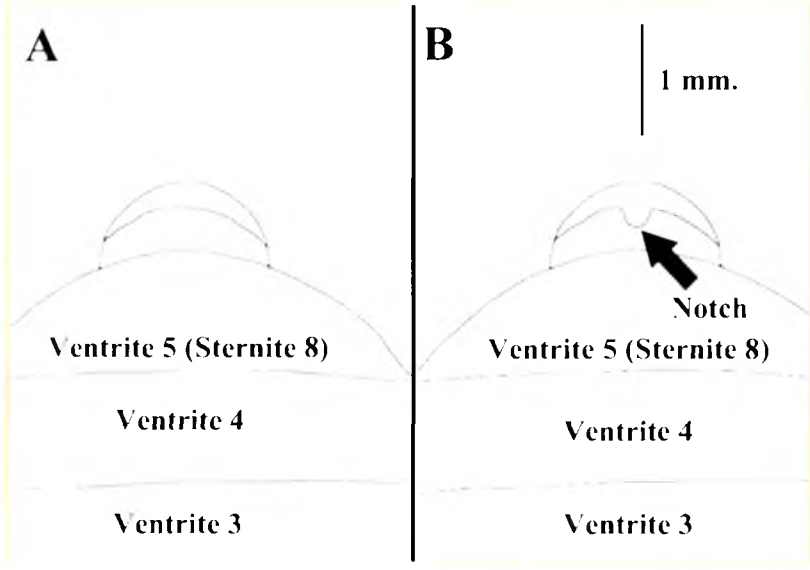

FIGURE 3. Posterior margin of 8th sternite in *Mesomorphus villiger* without semicircular median notch in female (A) and with semicircular median notch in male (B).

On the other hand, more than 50% of the beetles sexed by squeezing the abdomen died within three days. The extent of mortality of squeezed males were higher than that of the squeezed female beetles, which might be due to the damage arising from the squeezing out of genitalia, as the extruded male genitalia were not fully withdrawn.

ACKNOWLEDGEMENTS

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Habitat variation and dipteran diversity in Vadodara and its surroundings

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ABSTRACT: A study to record the Dipteran fauna of Vadodara and its surroundings was carried out from January 2005 till December 2007. The study was conducted in four selected habitats namely agricultural fields, community (public) gardens, fragmented habitats and residential sites. A total of 17 families belonging to 30 genera and 35 species were identified. Maximum number of species was represented by Muscidae (18%) followed by Stratiomyidae, Culicidae and Tabanidae representing 12%, Asilidae with 9%, Syrphidae and Dolichopodidae with 6%. The percentage composition of remaining families was much less. Population of Culicidae was maximum (38%) in residential areas due to presence of temporary, stagnant and dirty water pools made by humans and moderate vegetation, whereas all other families were maximum in fragmented habitats due to its ecological heterogeneity and stability. Almost all the species were found in all the four habitats, although the number spotted varied considerably. Shannon diversity (H) and eveness index (E) was significantly higher in fragmented habitats.

KEYWORDS: Diptera, Culicidae, Population and Diversity.

INTRODUCTION

Dipterans live in all habitats worldwide, including the subarctic and high mountains. They range in size from about 1 mm long midges to 3 inches long robber flies. Indeed no other insects present so great diversity of habit and habitats as the dipterans. Dipterans live in intimate association with other insects, animals and variegated flora interacts with each other and maintains equilibrium in the ecosystem.

Over 150,000 species have been described worldwide, but many more are awaiting description. Dipterans have been much more successful than their presumed sister-group, the Mecoptera (scorpionflies) which has less than 400 described species. Out of more than 130 families of dipterans all over the world, 87 families are found in India which is 12% of an estimated figure of the world species. Of which atleast

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6% still awaits discovery (Datta, 1998) remaining 6% of species holds 1075 genera with 6093 species. The Nematocera contains 22 families, Brachycera 15 families and Cyclorrhapha 50 families. The aquatic diptera mainly belong to Nematocera with 14 families, Brachycera with 4 families and Cyclorrhapha with 6 families. These aquatic species constitute 25% of the dipteran fauna. Out of total 6093 species 35% are endemic to India.

There is a scarcity of literature on habitat biodiversity hotspots regarding dipteran presence. Information on Dipteran biodiversity hotspots is lacking in general, and in the state of Gujarat in particular. This is the first comprehensive survey made on Dipterans of Vadodara district. It is situated in eastern part of the state of Gujarat in western India, located at 22°11' N latitude and 73°07' E longitude (Fig. 1).

Present study was undertaken with the purpose: (1) to record the biodiversity of the Order Diptera in and around Vadodara and (2) to find the extent of change of species composition from one habitat to another.

MATERIALS AND METHODS

Four different types of habitats were selected on the basis of ecological factors, flora, type of soil, surrounding environment and anthropogenic activities, to get an insight of the best possible insect diversity. Study was conducted during the period 2005 to 2007.

(a) Study sites (Tables 1a and 1b)

1. Agricultural fields (AF): all around Vadodara; 2. Community gardens (CG): Sayaji Baug and Lal Baug; 3. Fragmented habitat (FH): University campus and Laxmivilas Palace compound; 4. Residential areas (RA): New and old city area.

(b) Sampling method

Insects were collected throughout the year. Each study area was visited twice every month (7 am to 9 am and 5 pm to 7 pm) on the same day. At all the sites except in agriculture fields, quadrates of 10 m × 10 m were laid, while quadrates of 10 m × 5 m were laid in agricultural fields to decrease the sampling error. In Sweep net method each quadrate was walked until whole quadrate was covered several times during which net was swept. This was repeated after a gap of 10 minutes and 10 sweeps were performed each time. Hand collection was done in grass, shrubs, flowers, leaf litter, bare ground, tree bases, under stones, in field margins and tree trunks. All invertebrates were killed in the field using small quantities of 40% formaldehyde and later preserved in 70% ethanol for further examination.

(c) Identification

Insects collected were identified using keys available in Richards and Davies (1997); Borror *et al.* (1992); Leffroy (1909) and Ananthkrishnan and David (2004). The

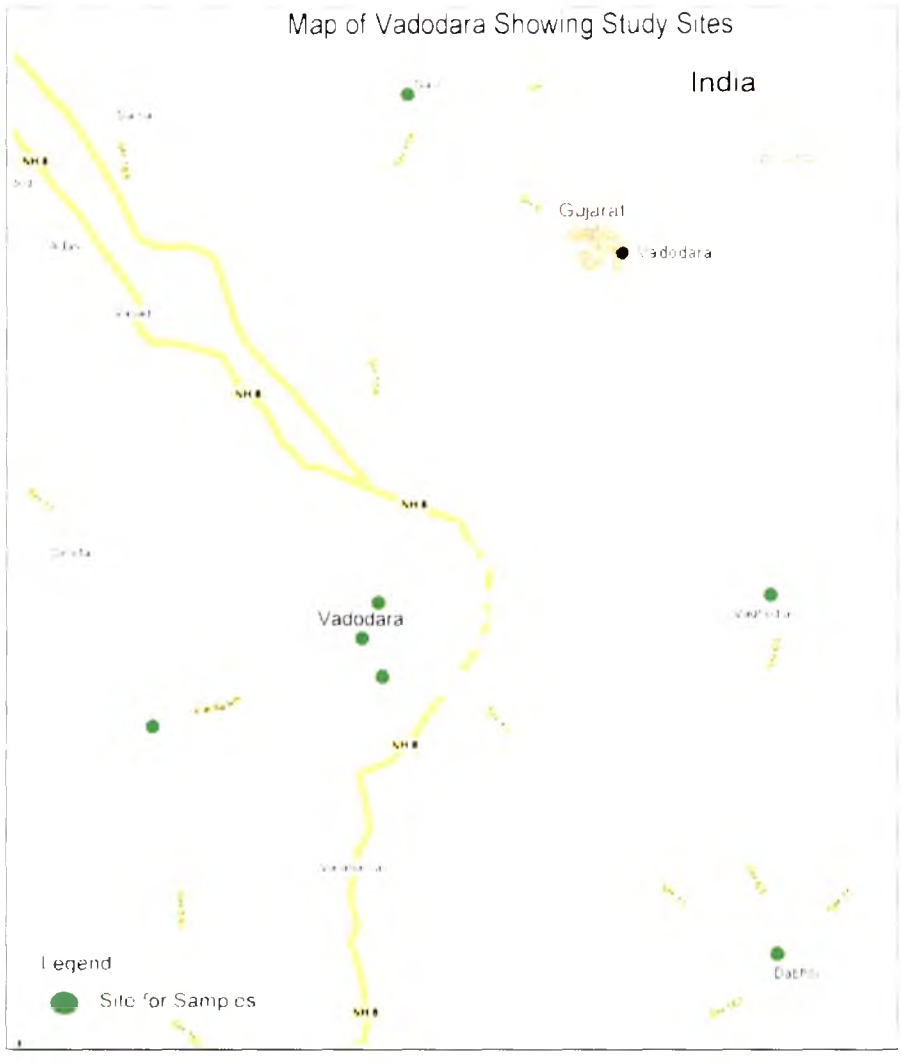

FIGURE 1. Map of Vadodara District showing study sites

identified material was confirmed from Entomology Division of Indian Agriculture Research Institute (IARI), PUSA, New Delhi.

(d) Data analysis

The raw data of all the sampled sites from the field diaries of three consecutive years were transferred on to an electronic format in spreadsheet layout (Microsoft excels). The data was finally analyzed to calculate important value indices from all

TABLE 1A. Checklist of Flora found in study sites of Vadodara

Study sites	Flora
Agricultural fields of Vadodara	Hedges: *Euphorbia neriifolia, Lawsonia inermis, Annona squamosa, Clerodendrum inermis, Zizyphus mauritiana, Calotropis procera, Capparis cepiaria* Crop plants: *Brassica olerasea var. campestris, Zea maize, Solanum melongina, Ricinus communis Spinacia olerecea, Raphanus sativus* Trees: *Azadirechta indica, Mangifera indica, Tamarindus indicus, Moringa oleifera* Herbs and Shrubs: *Rosa chinensis, Hibiscus Rosa sinensis Amaranthus viridis, Medicago sativa, Oxalis corniculata*
Community gardens	Trees: *Ficus bengalensis, Azadirachta indica, Aegle marmelos, Butea monosperma, Polyalthia longifolia, Mangifera indica, Tabebuia spectabilis* Herbs, Shrubs, Creepers: *Caeselpenia pulcherrina, Commelina nudiflora, Tephrosia purpurea, Hibiscus lobatus, Bougainvillea, Ixora coccinea, Andropogon annulatus, Cynodon dactylon, Vinca rosea, Rosa chinensis. Lantana camara, Canna indica, Nerium odorum, Ixora rubiacea, Tecoma stans, Oxalis corniculata, Mimosa pudica* etc.
Fragmented habitats	Trees: *Ficus benghalensis, Santalum album, Couroupita guianensis, Polyalthia longifolia, Saraca indica, Tamarindus indicus, Azadirahcta indica, Butea monosperma* Herbs, Shrubs, Creepers: *Nerium odorum, Hibiscus populineus, H. syriacus, Michelia champaca, Portulaca oleracea, Canna species, Viola odorata, Tamarix gallica, Tridax procumbens, Melilotus indica, Sida acuta, Cassia siamea, Acacia nilotica, Zizyphus jujube, Zornia diphylla, Oxalis corniculata* etc.
Residential areas	Trees: *Polyalthia longifolia, Ficus religiosa, Azadirechta indica, Livistona chinensis, Terminalia catappa, Alstonia scolaris* Herbs, Shrubs, Creepers: *Rosa chinensis, Ixora coccinea, Euphorbia neriifolia, Ocimum Sanctum, Vinca rosea, Zizyphus jujube, pothos, Nerium oleander, Jasminum sambac, Calotropis procera, Quisqualis indica Thevetia peruviana, Caeselpinia crista, Oxalis corniculata* etc.

Source: Sharad (1967)

the sampling sites. The diversity indices were calculated by Species diversity and richness version 2.65 (Henderson, 2003). The richness of species within habitats was calculated using Shannon–Weiner index (H) of alpha diversity index (H = Pi logeP). For measuring extent of change in species, from one habitat to another Whitaker's, and Wilson's were calculated — Whittaker index $\beta w = S/\alpha - 1$

$$\text{Wilson index } \beta_T = g(H) + l(H)/2\alpha$$

TABLE 1B. Description of study sites

Sr. no.	Study sites	Distance and Direction from Vadodara	Area in hectare	Soil type	Type of site
Agricultural fields					
1	Padra	15 Kms. in the southwest	3.23	Yellow sandy loam	Cultivated fields with large trees and hedges
2	Waghodia	15 Kms. on the east	4.50	Medium black	
3	Savli	25 Kms. on the north	2.50	Yellow sandy loam	
4	Dabhoi	30 Kms. on the southeast	4.80	Medium black	
Community gardens					
1	Lal Baug		3.6	Yellow sandy loam	Garden with pond & swimming pool
2	Sayaji Baug		40	Deep black , yellow sandy loam	Garden with river Vishwamitri passing through it
Fragmented habitats					
3	The M. S. University Campus		48	Yellow sandy loam	Buildings, Botanical garden, Cricket ground, Dense vegetation inside and surrounding the campus
4	Laxmivilas palace compound		286	Deep black	Palace with Dense vegetation and play ground.
Residential area		Within Vadodara city			
5	Ellora park		90	Yellow sandy loam	Construction sites, roads and pavements, residential houses. Patches of vegetation in private compounds
6	Navabazaar		85	Deep black	

Source: Municipal Corporation of Vadodara, Park and Garden Department, Sayajibaug

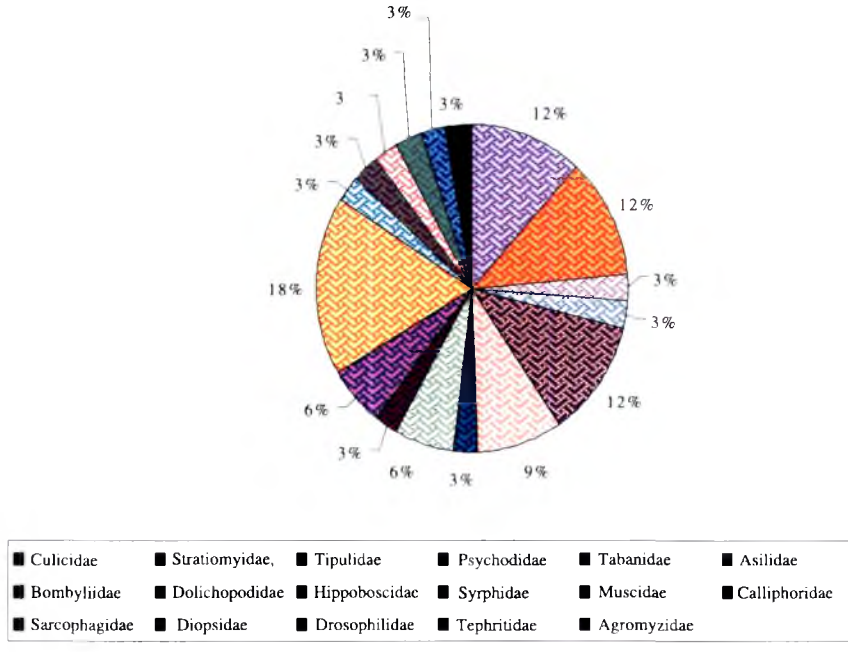

FIGURE 2. Overall percentage composition of Dipteran families in Vadodara.

Results

From a total of 2,716 individuals collected from the study site we found 17 families belonging to 30 genera and 35 species of Dipterans (Tables 2 and 3). Muscidae was represented by maximum number of species (18%) followed by Stratiomyidae, Culicidae and Tabanidae (12%), Asilidae with 9% and Syrphidae, Dolichopodidae with 6%. The percentage compositions of remaining families were observed to be much less (Fig. 2). Of all the individuals collected, fragmented landscape and community gardens accounted for 32% and 24% respectively while Agricultural fields and Residential areas contributed 26% and 18% respectively (Fig. 3). Culicid was most mportant at residential sites where it contributed 38% of the total dipteran population. At other sites, its contribution was not significant. The dominant taxon in this family was *Culex* species. The family Muscidae dominated the samples at all sites except esidential areas, contributing 38% at fragmented habitats and 27%, 26% and 9% at agricultural fields, gardens and residential areas respectively. It was represented by 6 species from 4 genera, Musca(3), Pycnosoma(1), Stomoxys(1) and Ochromyia(1) Table 3). The most important taxa were *Musca domestica*, *M. conducens*, *M. vicinia* ecorded the highest abundance at agricultural fields where as *Stomoxys calcitrans* was most abundant at Fragmented habitat. Family: Hippoboscidae was not prominent; t contributed only 1% of total population (Figs 4 and 5).

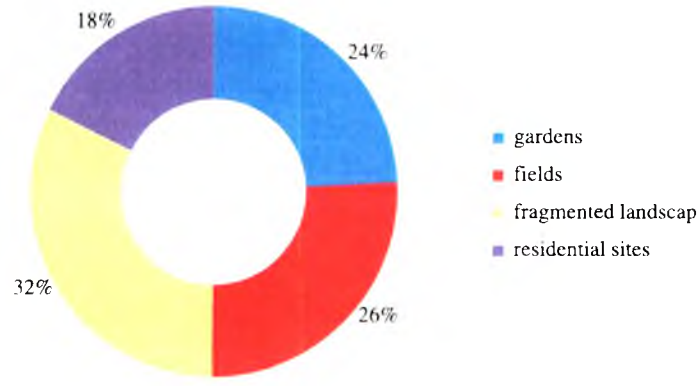

FIGURE 3. Percentage population of Dipteran species in various study sites.

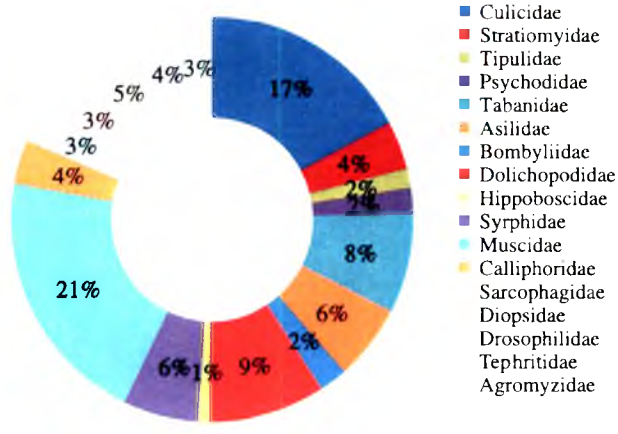

FIGURE 4. Percentage composition of individuals in all families.

Diversity indices

Out of all the insect orders, Diptera is one of the order, whose species are equally distributed in all the study sites but as the population of species differs, evenness also varies amongst the sites. Shannon diversity (H) was significantly higher in fragmented habitats as compared to other sites. The evenness index (E) followed the same trend. As shown in the Table 4, H value for fragmented habitat is maximum (3.45) and evenness index (E) (0.97) is also maximum. Minimum H value is for residential areas (3.14) with minimum evenness (0.88) and maximum dominance (0.11). Wilson Schmida test, however, did not reveal any significant dissimilarity between the pairs of sites. The values of Whittaker's and Wilson-schmida index for Beta diversity was zero as almost all the species were found in all the four habitats, although the number spotted varied considerably.

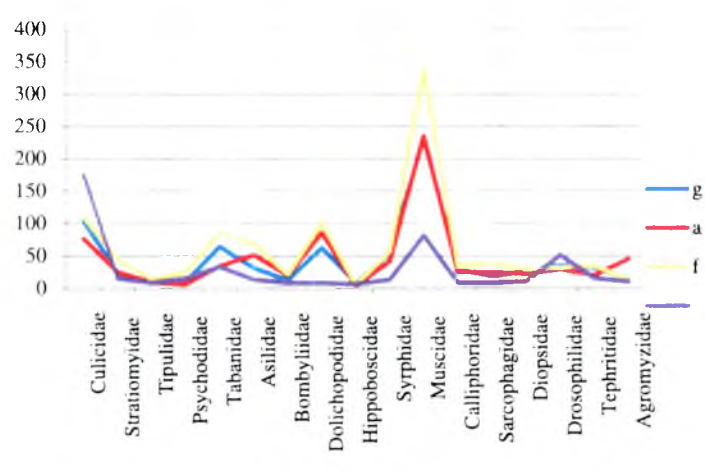

FIGURE 5. Number of individuals of all families in various study sites.

TABLE 2. Total Number of Families, Genera and Species.

Sub-order	Sr. No	Families	No. of genus	No. of species
Nematocera	1	Culicidae	3	4
	2	Stratiomyidae	4	4
	3	Tipulidae	1	1
	4	Psychodidae	1	1
Brachycera	5	Tabanidae	2	4
	6	Asilidae	3	3
	7	Bombyliidae	1	1
	8	Dolichopodidae	2	2
	9	Hippoboscidae	1	1
Cyclorrhapha	10	Syrphidae	2	2
	11	Muscidae	4	6
	12	Calliphoridae	1	1
	13	Sarcophagidae	1	1
	14	Diopsidae	1	1
	15	Drosophilidae	1	1
	16	Tephritidae	1	1
	17	Agromyzidae	1	1

DISCUSSION

Out of the 35 taxa recorded in this study, 6 (18%) were Muscids, dominated mainly by the genera Musca in terms of species richness. The diversity and evenness of species calculated by Shannon–Wiener function varied among the study sites. The significantly higher diversity in fragmented habitats is a reflection of its ecological heterogeneity and stability. The high evenness and low dominance index justify this

TABLE 3. Checklist of insects found in all study sites

Nos	Family	Nos	Species	A	CG	F	R
1	Culicidae	1	*Culex pipiens* Linnaeus, 1758	1	1	1	1
		2	*C. quinquefasciatus*	1	1	1	1
		3	*Anopheles quadrimaculatus* say, 1824	1	1	1	1
		4	*Aedes aegypti* Linn 1762	1	1	1	1
2	Stratiomyidae.	5	*Pachygaster* species	1	1	1	1
		6	*Hermatia illucens* Linn. 1758	1	1	1	1
		7	*Sargus metallinus* Fab. 1805	1	1	1	1
		8	*Wallacea argentea* Doleschall, 1858	1	1	1	1
3	Tipulidae	9	*Conosia irrorata* Wiedemann	1	1	1	1
4	Psychodidae	10	*Telmatoscopus* species	1	1	1	1
5	Tabanidae	11	*Tabanus lineola* Fabricius, 1794	1	1	1	1
		12	*Tabanus rubidus* Wiedemann, 1821	1	1	1	1
		13	*Tabanus striatus* Fabricius, 1787	1	1	1	1
		14	*Chrysops dispar*	1	1	1	1
6	Asilidae	15	*Philodicus femoralis* Ricardo, 1921	1	1	1	1
		16	*Promachus duvancelii*	1	1	1	1
		17	*Allocotasia aurata* Fabricius, 1794	1	1	1	1
7	Bombylidae	18	*Argyramoeba distigmata*	1	1	1	1
8	Dolichopodidae	19	*Condylostylus species*	1	1	1	1
		20	*Heteropsilopus/Sciapus Sp*	1	1	1	1
9	Hippoboscidae	21	*Hippobosca variegata* Megerle, 1803	1	1	1	1
10	Syrphidae	22	*Sphaerophoria scutellaris*	1	1	1	1
		23	*Helophilus bengalensis*	1	1	1	1
11	Muscidae	24	*Musca domestica* Linnaeus, 1758	1	1	1	1
		25	*M.conducens* Walker, 1859	1	1	1	1
		26	*M. vicinia*	1	1	1	1
		27	*Pyenosoma flaviceps*	1	1	1	1
		28	*Stomoxys calcitrans* Linnaeus, 1758	1	1	1	1
		29	*Ochromyia jejuna*	1	1	1	1
12	Calliphoridae	30	*Lucilia illustris* Meigen, 1826	1	1	1	1
13	Sarcophagidae	31	*Sarcophaga lineatocolis* Macq	1	1	1	1
14	Diopsidae	32	*Sphyracephala hearseyana*	1	1	1	1
15	Drosophilidae	33	*Drosophila melanogaster* Meigen, 1830	1	1	1	1
16	Tephritidae	34	*Dacus dorsalis* Hendel 1912	1	1	1	1
17	Agromyzidae	35	*Melanagromyza obtusa* Malloch 1914	1	1	1	1

1: Present. 0: Absent

TABLE 4. Species diversity and evenness in all the study sites

Diversity measure	Agriculture fields	Community gardens	Fragmented habitats	Residential sites
Shannon Diversity (H)	3.35	3.42	3.45	3.14
Species Richness Index (J)	0.94	0.96	0.97	0.88
Berger Parker	0.06	0.05	0.05	0.11
Species no.	35	35	35	35

situation. Higher the evenness higher is the diversity and lower is the dominance index (Victor and Ogbeibu, 1985). If overall diversity is used as a measure of community and ecosystem stability, the dipteran community of fragmented habitat must be considered more stable than other sites. The faunal similarity analysis of the four study sites showed that residential areas were significantly dissimilar from agricultural fields.

Damp and shady places of fragmented habitats were mostly occupied by *Pachygaster Sp., Sargus metallinus, Conosia irrorata, Sphyracephala hearseyana* and *Dacus/Bactrocera dorsalis*. Small, stagnant, temporary and dirty waterpools and moderate vegetation near human habitations in residential areas and agricultural fields were having *Culex pipiens, Culex quinquefasciatus, Anopheles quadrimaculatus* and *Aedes aegypti*. Some species like *Chrysops dispar, Tabanus rubidus, T. striatus, T lineola* and *Hippobosca variegata* were found on cattles near agricultural fields, fragmented habitats and residential areas. *Hermatia illucens, Helophilus bengalensis, Lucilia illustris, Sarcophaga lineaticollis* and *Drosophila melanogaster* were found hovering on decaying animal and vegetable matter around the fields, in fragmented habitats and residential areas. *Telmatoscopus albipunctatus* were found on damp walls of houses of residential areas and of fields. *Pycnosoma flaviceps* and *Lucilia illustris* was hovering on *Calotropis* in the university campus, Laxmivilas Palace compound, hedges of all agricultural fields, on sides of roads in new residential areas and margins of community gardens. *Stomoxys calcitrans* was commonly seen in the sun shine on fences, posts and boards in all the sites. Fields of *Cajanus cajan* were possessing *Melanagromyza obtuse, Allocotasia aurata* and *Philodicus femoralis* which were found on vegetation of fragmented habitats, agricultural fields and community gardens. All of these sites were having good population of aphids, jassids, grasshoppers, small beetles and hymenopterans.

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The genus *Nephele* Hübner (Lepidoptera : Sphingidae) from India with description of a new species

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ABSTRACT: The male and female genitalic features of three Indian species of genus *Nephele* Hübner viz., *didyma* (Fabricius), *hespera* (Fabricius) and *joiceyi* Clark have been illustrated in detail along with the description of a new species i.e., *kitchingi*. A key to the known Indian species of this genus has also been formulated.

KEYWORDS: *Nephele*, Sphingidae, India, new species

INTRODUCTION

Hawk moths belonging to the genus *Nephele* Hübner have been collected from different localities of Himachal Pradesh, Karnataka, Punjab and Uttarakhand. Though they appeared to belong to a single species, but after close examination of morphological characters including male and female genitalic structures, they were grouped under four distinct species. Out of these, all the three known species viz., *didyma* (Fabricius), *hespera* (Fabricius) and *joiceyi* Clark have been identified and the fourth one has been designated as a new species after following the relevant literature (Cotes & Swinhoe, 1887; Hampson, 1892; Clark 1922–1938; Bell & Scott, 1937; D'Abrera, 1986; Zhu & Wang, 1997; Beck & Kitching, 2000; Kitching & Cadiou, 2000; Pittaway & Kitching, 2000) and by comparisons with the identified collections of the National museums. The male and female genitalic features of all the species have been studied and illustrated in detail and included while formulating the keys to the species. The terminology by Klots (1970) has been followed for naming genitalic parts. All these species completely conform to the characterization of the genus and form a natural group. The genus is characterized by the presence of a comb of stout spines on short tibial spurs of mid and hind tibiae.

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Genus *Nephele* Hübner

Hubner, 1822, *Verz. bek. Schmett.*, **1822**: 133; Hampson, 1892, *Moths India*, **1**: 108; Rothschild and Jordan, 1903, *Novit. Zool.*, **9**: 550; id., 1907, In Wytsman, *Gen. Ins.*, **57**: 104; Bell and Scott, 1937, *Fauna British India, Moths*, **5**: 324–325.

Type species: *Sphinx didyma* Fabricius

Distribution: Oriental and Palaearctic regions.

Diagnosis: Labial palpus upturned, second segment widened from base to apex, rounded, incrassate at end; inner surface of first segment carinate ventrally. Proboscis long, having fine hair at each side of base. Antenna not incrassate distally in male, slightly clubbed in female, end segments long, roughly scaled. Forewing with distal margin entire; basal one-fourth portion of anal vein forked; Cu_2 from middle of cell; Cu_1 before lower angle; M_3 from angle; M_2 from below middle of discocellulars; M_1 from upper angle or from base of common stalk of R_5 and R_4; $R_{(3+2)}$ well before upper angle; R_1 from well beyond middle of cell; discal cell half the length of wing. Hindwing with both anal veins present; 2A forked at base; Cu_2 beyond middle of cell; Cu_1 from well before lower angle; M_3 from angle of cell; M_1 and Rs from upper angle or stalked; Sc + R_1 anastomosing with cell before middle; discal cell less than half the length of wing. Legs with mid tibia having one pair and hind tibia two pairs of tibial spurs; short spur of mid tibia and short terminal one of hind tibia with a comb of stout spines; mid and hind tarsal comb strongly developed. Male genitalia with uncus slender, simple, curved; gnathos narrow; valva with friction scales; saccular projection sharply curved with narrow apex; aedeagus having two dentate processes, proximal one long. Female genitalia with longitudinal signum, well sclerotized genital plate.

Nephele didyma (Fabricius)

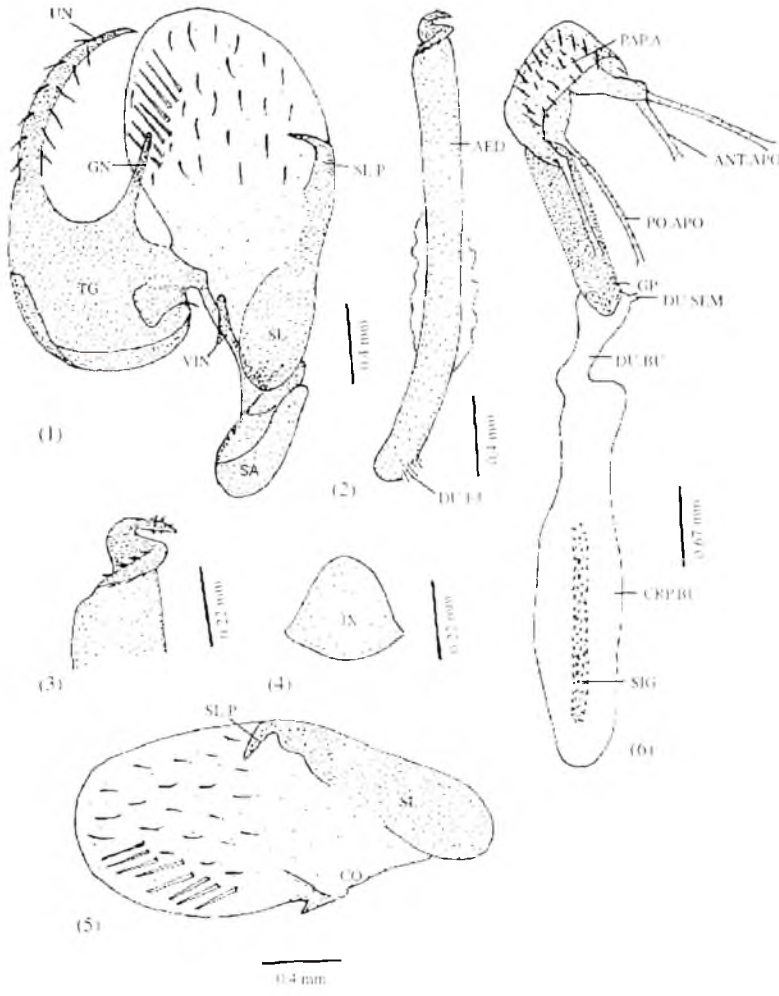
Sphinx didyma Fabricius, 1775, *Syst. Ent.*, **1775**: 543.

Nephele didyma Fabricius: Rothschild and Jordan, 1903, *Novit. Zool.*, **9**: 554; Seitz, 1929, In Seitz's *Macrolep.*, **10**: 554; Bell and Scott, 1937, *Fauna British India, Moths*, **5**: 325–327.

Zonilia morpheus, Cramer, 1777, *Pap. Exot.*, **2**: 84; Walker, 1856, *List Specimens Lepid. Insects. Br. Mus. London*, **8**: 194.

Male genitalia

Uncus narrow, long, moderately sclerotized, sickle-shaped with pointed tip, setosed with few setae; gnathos simple, triangular basal area, distal portion narrow with rounded tip, semi-sclerotized; tegumen broad, inverted U-shaped, semi-sclerotized, almost 2X length of vinculum; vinculum quite narrow, semi-sclerotized ending into rounded, slightly sclerotized saccus; juxta campanulate, slightly sclerotized; transtilla triangular, semi-membranous with one arm produced. Valva simple, ovoid, just extending up to uncus, semi-membranous, setosed; costa narrow, semi-membranous; sacculus well differentiated; saccular projection broad at base, falcate with narrow distal end, tip pointed; distal portion of valva semi-membranous, setosed, outer wall armed with sclerotized, long, narrow friction scales. Aedeagus narrow, both of its



FIGURES 1–6. *Nephele didyma* (Fabricius): 1. Male genitalia – lateral view; 2. Aedeagus; 3. Aedeagus – distal and (enlarged); 4. Juxta – ventral view; 5. Valva – ventral view; 6. Female genitalia.

walls equally sclerotized, a sclerotized sheath present at one-third portion; distal end with bicuspid spur having unequal arms, bearing small denticles; vesica without any cornuti.

Female genitalia

Corpus bursae small, quite oblong, membranous, signum longitudinal having parallel rows of denticles present in the middle of corpus bursae; ductus bursae membranous,

basal two-third portion guarded by moderately sclerotized genital plate; ductus seminalis originating from anterior end of genital plate; anterior apophyses shorter than posterior ones; posterior apophyses narrow at base; both pairs dilated, membranous apices; papilla analis broad, densely fringed with fine setae.

Wing Expanse: Male: 66–76 mm; Female: 70 mm.

Material Examined: Himachal Pradesh: Bahli, 13.VI.2000, 1♂; Kangra, 12.VI.1998, 1♂; Karsog, 21.VI.2001, 1♂; Macleodganj, 16.VI.1998, 1♀; Manali, 14.VI.2004, 1♂; Mandi, 12.VI.1999, 1♀; Paonta Sahib, 16.V.1993, 1♂; Tanyar, 26.VII.1998, 4♂♂. Karnataka: Jog Falls, 16.XI.2003, 1♂. Punjab: Patiala, 02.XI.1984, 1♀; 23.X.1991, 3♂♂. Uttarakhand: Dehradun, 01.VII.2004, 1♂; FRI, 24.IV.1999, 1♂; Kempty Falls, 02.VII.2004, 1♀; Mandal, 22.VI.2003, 1♂; Mussourie, 05.05.1993, 1♂.

Distribution: Throughout India; China; Indonesia; Malaysia; Myanmar; Nepal; Philippines; Sri Lanka; Thailand; Vietnam.

Remarks: The species under reference is distinct due to the presence of a pair of silvery spots at the end of discal cell.

Nephele kitchingi n. sp.

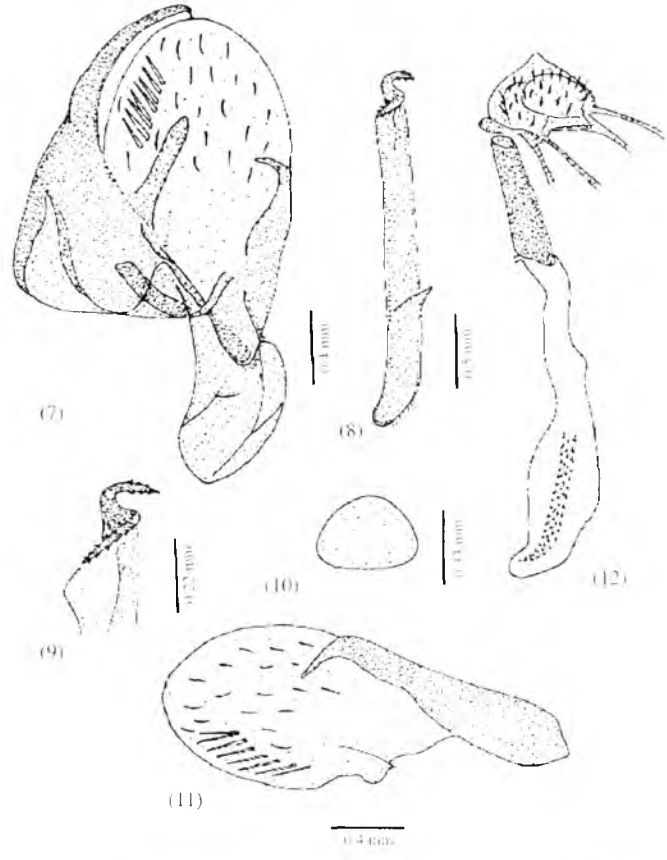
Head with vertex and frons covered with brown scales. Antenna with scape and basal half of flagellum brown, distal half white. Eyes golden brown. Labial palpus upturned, surpassing middle of frons; second segment incrassate; underside fringed with white scales.

Thorax, collar and tegula furnished with olive green scales, collar edged with white; underside paler. Forewing with ground colour olive green; a white speck at base of cell; another at end of cell; only submarginal line strongly bent in middle, marginal area beyond it darker; fringe fuscous brown; underside paler, a white speck at end of discal cell; two postmedial lines; outer area fuscous; basal one thirds of anal vein forked; M_1 (R_5, R_4) stalked; R_1 from three fourths of cell. Hind wing with ground colour olive green except costal area; outer area darker with a tinge of russet; fringe pale brown; underside paler having two faint lines; M_2 from above middle of discocellulars; M_1 and Rs from upper angle, not stalked; $Sc+R_1$ anastomosing with cell before middle. Legs dressed with pale brown scales; mid tibia having one pair and hind tibia having two pairs of tibial spurs, inner ones long; tarsi spinose.

Abdomen decorated with olive-green scales; lateral dark bands on segments three to five; remaining segments completely ringed with black.

Male genitalia

Uncus long, narrow, sickle-shaped ending into spined tip, slightly, semi-sclerotized; gnathos simple, nearly straight, semi-sclerotized, tip narrow; tegumen broad, slightly sclerotized, inverted U-shaped, almost 2X length of vinculum; vinculum narrow ending in broad U-shaped saccus, slightly sclerotized; juxta ovoid, semi-membranous, proximal end not notched. Valva simple, not even extending up to level of uncus, short,



FIGURES 7–12. *Nephele kitchingi* n. sp. 7. Male genitalia – lateral view; 8. Aedeagus; 9. Aedeagus – distal and (enlarged); 10. Juxta – ventral view; 11. Valva – ventral view; 12. Female genitalia.

ovoid; costa narrow; sacculus well defined, semi-sclerotized, saccular projection broad at base, distal end strongly bent, narrow with pointed tip; distal end of valva rounded, setosed, outer wall armed with sclerotized, narrow, long friction scales. Aedeagus narrow of moderate length, semi-sclerotized, proximal end produced; a sclerotized-sheath projection near one-third portion, distal end having a bicuspid spur, both arms of equal length, studded with small denticles; vesica without any armature.

Female genitalia

Corpus bursae short, membranous, oblong, walls corrugated; longitudinal, percurrent signum; ductus bursae membranous, lower half guarded by moderately sclerotized genital plate; ductus seminalis originating from anterior end of genital plate; anterior

apophyses shorter than posterior ones; posterior apophyses narrow at base; both pairs having membranous apices; papilla analis broad, setosed with equal sized setae.

Wing Expanse: Male: 64–72 mm; Female: 76 mm.

Material Examined: Holotype: Himachal Pradesh: Shimla, 13.VI.1999, 1♂.

Paratypes: Himachal Pradesh: Shimla, 13.VI.1999, 3♂♂, 4♀♀. Uttarakhand: Mussourrie, 05.V.1993, 1♂; 06.VI.1993, 1♀.

Distribution: India: Himachal Pradesh, Uttarakhand

Remarks: This species is closely allied to *hespera* (Fabricius) as far as the presence of white speck at the end of discal cell and venation is concerned, but distinct from all the species due to the absence of all six wavy antemedial, medial and postmedial lines. The other distinct features include broader gnathos, ovoid juxta and long uncus extending beyond the level of valva in male genitalia and short, oblong corpus bursae having wrinkled walls and membranous apices of apophyses in female genitalia.

Etymology

The species has been named in the honour of Dr. I. J. Kitching, the well known Lepidopterist.

Nephele hespera (Fabricius)

Sphinx hespera Fabricius, 1775, *Syst. Ent.*, *1775*: 546.

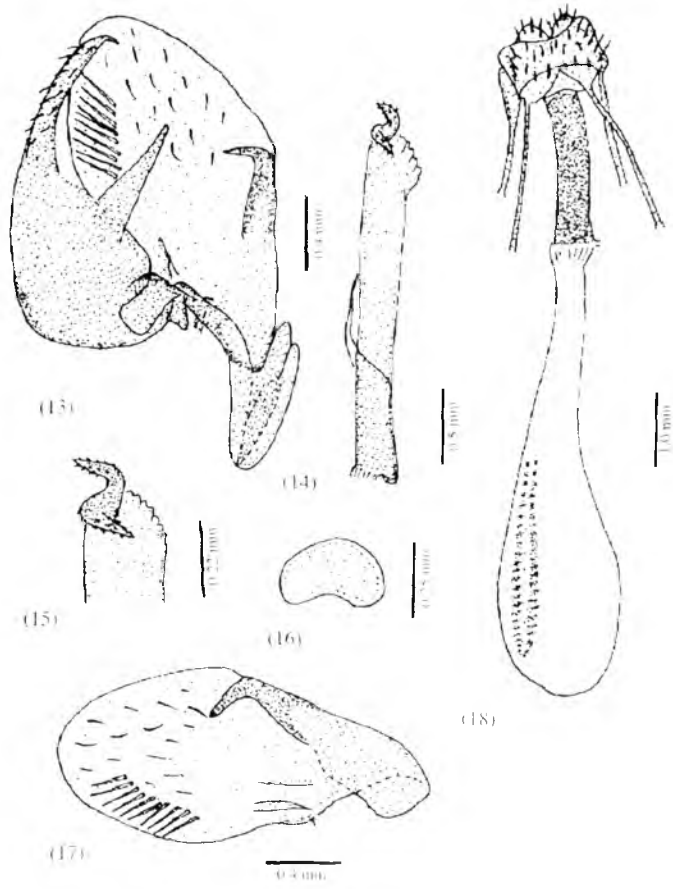
Nephele hespera Fabricius: Hübner, 1822, *Verz. bek. Schmett.*, **1822**: 133; Hampson, 1892, *Moths India*, **1**: 108; Bell and Scott, 1937, *Fauna British India, Moths*, **5**: 328.

Male genitalia

Uncus narrow, moderately sclerotized, sickle-shaped with pointed tip; gnathos narrow, distal portion quite narrow, semi-sclerotized, tip blunt; tegumen broad, slightly sclerotized, almost 2X length of vinculum; vinculum narrow, ending into U-shaped saccus, slightly sclerotized; juxta simple dome-shaped, proximal end notched, slightly sclerotized; transtilla triangular. Valva of moderate size, just extending beyond level of uncus; costa narrow; sacculus well defined, saccular projection broad at base, distal half strongly bent, narrow with pointed tip, moderately sclerotized; distal half of valva broad, semi-membranous, setosed, outer wall studded with well sclerotized multiple long projections. Aedeagus narrow of moderate length, proximal end produced; a sclerotized sheath extension near one-third portion of aedeagus, distal end having a bicuspid spur, both extending in opposite directions and bearing small denticles; vesica without any cornuti.

Female genitalia

Corpus bursae small, oblong, membranous; signum longitudinal, represented by parallel rows of small denticles running nearly entire length of corpus bursae; ductus bursae long, narrow, membranous, basal half guarded by well sclerotized genital plate; ductus seminalis originating from anterior end of genital plate; anterior apophyses half



FIGURES 13–18. *Nephele hespera* (Fabricius) 13. Male genitalia – lateral view; 14. Aedeagus; 15. Aedeagus – distal end (enlarged); 16. Juxta – ventral view; 17. Valva – ventral view; 18. Female genitalia.

length of posterior ones; posterior apophyses narrow, long; both pairs having slightly dilated apices; papilla analis small, fringed with fine setae.

Wing Expanse: Male: 62–72 mm; Female: 74–76 mm.

Material Examined: Himachal Pradesh: Bahli, 13.VI.2000, 1♂; Chowki, 22.VI.2001, 3♂♂; Kharapathar, 23.VI.2001, 1♂; Kotkhai, 12.VI.2000, 2♂♂; Kursog 21.VI.2001, 1♂; Manali, 13.VI.2004, 1♂; Manikaran, 15.VI.2004, 1♂; Raison, 16.VI.2004, 1♂, 1♀; Renuka, 09.VIII.1998, 1♂; Sarkaghat, 25.VII.1998, 1♂; Shimla, 13.VI.1991, 1♂, 2♀♀; 06.VII.1998, 1♂; Tanyar, 26.VII.1998, 2♂♂, 3♀♀. Karnataka: Bhagamandalam, 25.XI.2003, 1♂; Jog Falls, 18.VII.1991, 1♀

Uttarakhand: Dehradun, FRI, 22.IV.1999, 1♂; Kempty Falls, 02.VII.2004, 1♂; Mandal, 22.VI.2003, 1♀; Mussourie, 03.VII.2004, 1♂; Srinagar, 26.VI.2003, 1♂.

Distribution: Throughout India; Afganistan; Australia; China; Indonesia; Myanmar; Nepal; Sri Lanka; Pakistan; Thailand; Vietnam.

Remarks: This species can be differentiated from the type species *didyma* (Fabricius) on the basis of origin of veins M_1 and Rs in hind wing, presence of white speck at end of discal cell in forewing and distinct juxta and corpus bursae. It is widely distributed throughout India.

Nephele joiceyi Clark

Clark, 1937, *Proc. New England Zoological Club*, **16**: 27–39.

Male genitalia

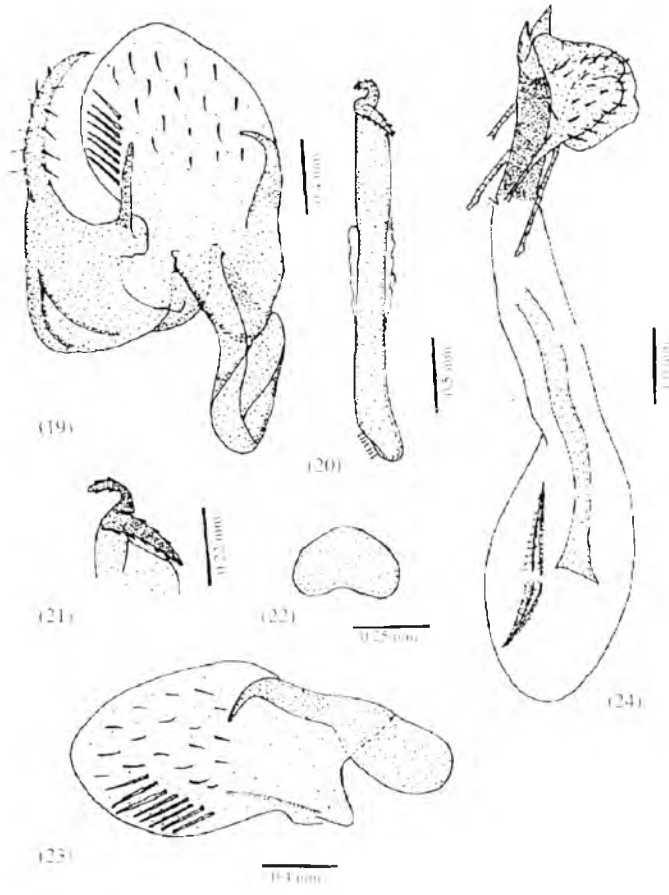
Uncus narrow, long, sickle-shaped, moderately sclerotized with spined tip; gnathos simple, moderately sclerotized narrow, straight, distal half quite narrow with rounded tip; tegumen broad, inverted V-shaped, slightly sclerotized, almost 2X length of vinculum; vinculum narrow, ending into rounded saccus; juxta simple dome-shaped, proximal end notched, semi-membranous; transtilla bursiform. Valva simple, just extending level of uncus, ovoid; costa narrow; sacculus well differentiated; saccular projection broad at base, narrowing towards tip, distal half falcate, distal end blunt; cucullus and valvula not differentiated, distal half of valva rounded, semi-membranous, outer wall studded with narrow sclerotized long, friction scales. Aedeagus narrow, of moderate length; proximal end produced, distal end armed with bicuspid spur with unequal arms, extending into opposite directions, beset with small denticles; vesica without any cornuti.

Female genitalia

Corpus bursae small, ovoid, membranous; signum longitudinal with both ends narrow, having parallel rows of small denticles; well sclerotized duct extending up to anterior half of ductus bursae; ductus bursae membranous, basal one-third portion guarded by well sclerotized genital plate; ductus seminalis from anterior end of genital plate; anterior apophyses more than half length of posterior ones, apex slightly dilated; posterior apophyses long, narrow slightly constricted near apex; papilla analis small, broad, setosed with fine setae.

Wing Expanse: Male: 64–72 mm; Female: 70 mm.

Material Examined: Himachal Pradesh: Kangra, 12.VI.1998, 1♂; Sarkaghat, 25.VII.1998, 1♀; Shimla, 13.VI.1991, 5♂♂; Tanyar, 26.IX.1983, 1♂, 1♀. Uttarakhand: Dehradun, FRI, 24.IV.1999, 1♂, 1♀; Mussourie, 05.V.1993, 2♂♂; 06.VI.1993, 1♂; 01.VI.1998, 1♂.



FIGURES 19–24. *Nephele joiceyi* Clark 19. Male genitalia – lateral view; 20. Aedeagus; 21. Aedeagus – distal and (enlarged); 22. Juxta – ventral view; 23. Valva – ventral view; 24. Female genitalia.

Distribution: India: Himachal Pradesh, Uttarakhand; South East Asia.

Remarks: The collection of this species from Himachal Pradesh and Uttarakhand is its additional record from India. This species is distinct from all the other congeneric species due to the presence of a black discoidal speck

Key to the Indian species of genus *Nephele* Hübner:

1. Male genitalia with juxta campanulate, female genitalia with corpus bursae larger than ductus bursae; forewing with two silvery spots at end of discal cell; hind wing with veins M_1 and Rs stalked from upper angle of cell ..... *didyma* (Fabricius)

Male genitalia with juxta ovoid or bean shaped; female genitalia with corpus bursae shorter than ductus bursae; forewing with one white or black speck at end of discal cell; hind wing with veins M_1 and Rs from upper angle of cell, not stalked 2

2. Female genitalia with corpus bursae ovoid, walls smooth; apophyses with apices not membranous; forewing with six antemedial, medial and postmedial lines; M_2 from middle of discocellulars 3

Female genitalia with corpus bursae oblong having corrugated walls; apophyses with apices membranous; forewing without any antemedial, medial and postmedial lines; M_2 from above middle of discocellulars ..... *kitchingi* n.sp.

3. Male genitalia with uncus having pointed tip; female genitalia with posterior apophyses having dilated apices; signum not pointed at both ends; forewing with a white speck at end of discal cell; M_1 from upper angle of cell, (R_5, R_4) stalked. ..... *hespera* (Fabricius)

Male genitalia with uncus having spined tip; female genitalia with posterior apophyses having constricted apices; signum pointed at both ends; forewing with a black speck at end of discal cell; M_1 (R_5, R_4) stalked ..... *joiceyi* Clark

Abbreviations

AED – Aedeagus; ANT.APO – Anterior apophyses; CO – Costa; CRP. BU – Corpus bursae; DU.BU – Ductus bursae; DU. EJ – Ductus ejaculatoris; DU. SEM – Ductus seminalis; GN – Gnathos; G.P – Genital plate; JX – Juxta; PAP. A – Papilla analis; PO. APO – Posterior apophyses; SA – Saccus; SIG – Signum; SL – Sacculus; SL. P – Sacculer Projection; TG – Tegumen; UN – Uncus; VES – Vesica; VIN – Vinculum.

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Rate of development of Head Capsule Width (HCW) of various larval instars of *Leucinodes orbonalis* Guenee (Lepidoptera: Pyraustidae)

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ABSTRACT: Biometrical analysis of *Leucinodes orbonalis* (brinjal shoot and fruit borer) showed growth of its head capsule width in successive larval instars in a geometric progression revealing the applicability of Dyar's law. This was indicated by mean observed values (0.28–1.482) and calculated values (0.28–1.628) of head capsule width. The progression factors were also found close to each other. The coefficient of determination (r^2 = .988) also indicated a high predictability of head capsule width in geometric pattern with linear regression line capturing 99–100 percent of variation in head capsule width.

KEYWORDS: *Leucinodes orbonalis*, brinjal shoot and fruit borer, head capsule, larvae and geometric progression.

INTRODUCTION

Life cycles of insects vary greatly among ametabolous, hemimetabolous, and holometabolous groups. There is also great variation in the number of moults in various species. Ametabolous insects moult 10, 20 and even up to 60 times before attaining sexual maturity, while hemimetabolous species most commonly have 4–6 nymphal moults before attaining adult stage. Holometabolous insects may have more than 2 instars. The growth of individual insect proceeds in a progressive manner through out the immature period of development, although the rate of growth can vary depending on a variety of factors such as moulting frequency, temperature, and nutrition. Insect body size can be estimated by using simple linear measures, such as head capsule width or body length. Since the publication of the pioneering works of Hutchinson and Tongring (1984), one of the most widespread uses of the width of

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head capsule is for identifying and determining the number of immature stages. The objective of this study was to determine the number of instars of *Leucinodes orbonalis* based on the distribution of head capsule widths from laboratory observations.

MATERIAL AND METHODS

A study was conducted to standardize the mass rearing of *L. orbonalis* on brinjal in laboratory between March–October, 2010. Immatures of *L. orbonalis* were reared until adult emergence under controlled ecological conditions at 25 ± 2° C and 70 ± 5% R.H and ten replicates for each stage were used. The life cycle of *L. orbonalis* took about a month to complete. The newly hatched larvae were reared in plastic containers covered by muslin cloth with fresh food replenished daily. At intervals of 2–4 days, the larvae were immobilized by keeping them in freezer for 2–4 minutes according to their size and later, measurements of width of head capsules were made with an occular micrometer with a correction to nearest 0.01 mm. After measurements, these immobilized immatures were reared and head capsule width in successive instars was recorded like wise. Data so obtained were transformed into logarithms and subjected to a linear regression analysis.

RESULTS

The widths of head capsule measured from larvae fell into five distinct classes suggesting five larval instars viz. instar I (mean ± SD: 0.28 ± .036), instar II (0.478 ± 0.131), instar III (0.764 ± .074), instar IV (1.106 ± 0.18) and instar V (1.482 ± 0.154). Average growth ratio and the slope of regression line indicated that larval growth followed a geometric pattern (Fig. 1). The growth factor is calculated by dividing the linear measure of size for one instar by that measure of size for the previous instar. The present study points to a growth ratio of 1.5.

Several mathematical models have been used to describe linear measurement of head capsule in successive instars. The present study utilized the semi log regression model to estimate the number of larval instars using linear equation $Y = a + bX$ where Y is a measure of size, X is the instar number, a and b are constants. This relation has also been termed Brooks Dyar rule (Hutchinson and Tongring, 1984). Therefore, a plot of log transformed size measurements against instar number revealed a straight-line relation. The correlation co-efficient (r) of five instars was 0.994 and co-efficient of determination (r^2) was = 0.988. A comparison of the calculated and the observed width did not reveal any significant difference and therefore, it can be concluded that no ecdyses were overlooked.

DISCUSSION

Dyar's rule (1890) states that larval growth progresses geometrically and by a relatively constant ratio. Dyar's rule also predicts that a linear measure of size increases by a constant factor from one instar to the next. The growth factor is

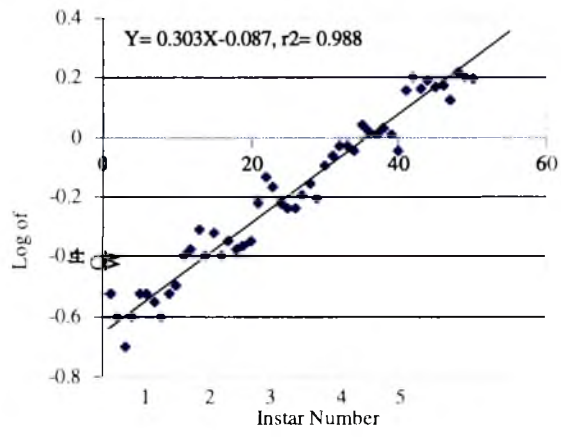

FIGURE 1. Log of head capsule width (HCW) of *L. orbonalis* as a function of instar number. $Y = 0.303X-0.087$. Y = Head capsule width (in mm). X = Larval instars.

calculated by dividing the linear measure of size for one instar by that measure of size for the previous instar. This produced a growth ratio for each larval moult. According to Dyar's rule growth ratio for head capsule of lepidopteran insects is commonly $\approx$1.4. The present study points to a growth ratio of 1.5 and this does not deviate markedly from 1.4 generally observed for insects. This difference may be attributed to the effects of biotic and abiotic factors.

Although Dyar's law has been widely used in entomological studies, the progression in the size of sclerotized body parts is not always constant and can be influenced by abiotic and biotic factors such as temperature and food. Also, for holometabolous insects it is not the number of instars that determines, rather a threshold size determines a particular last larval instar (Nijhout, 1975) and within this last instar, an instar weight determines when metamorphosis occurs (D'Amico *et al.*, 2001). Observations on the increase in width of the head and the frons in successive larval instars indicate that *L. orbonalis* has five larval instar supported by Jethva and Vyas (2009). Earlier studies have also reported that nutritional quality of tomato has also not inhibited the five larval instars of *L. orbonalis* as supported from the observations of Jethva and Vyas (2009). Although, Dyar's rule defines growth of HCW for lepidopteron larvae, studies have been reported for its applicability in other insect group like Orthoptera (Singh *et al.*, 1988; Meena and Singh, 2009). The present study of HCWs in *L. orbonalis* suggested that Dyar's law could be used satisfactorily to check the number of moulting during larval life directly associated with feeding and increase in size.

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Studies on life cycle parameters of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida)

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ABSTRACT: The life cycle parameter studies of leafhopper, *Empoasca devastans* Distant indicated that first instar recorded maximum duration (2.58 ± 0.57 days), followed by the second instar (2.14 ± 0.81 days), third instar (2.03 ± 0.63 days) and fourth instar (1.84 ± 0.80 days). Newly hatched nymphs were transparent with greenish yellow in colour and were very delicate. The second instar nymph was transparent with greenish yellow body, slightly differing in morphology from first instar with regards to eyes, while third instar nymph was found almost similar to second instar in its appearance except the colour. The last and fourth instar nymphs were stout with dark yellowish green colour and developed wing pads, which reached up to middle of abdomen. The total nymphal period varied from 5–16 days with an average of 8.59 days which was less than the adult longevity of 13.37 ± 5.17 days. The average male longevity was 21.38 ± 5.39 days, while female longevity was 23.19 ± 4.86 days indicating that females survived longer than males. Per cent mortality was highest in first instar nymphs. Sex ratio was biased towards female (1:1.31 male:female). The egg stage could not be located. The study is useful in targeting susceptible developmental stages for maximum efficacy of insecticides.

KEYWORDS: cotton, leafhopper, *Amrasca biguttula biguttula*, life cycle.

Cotton is an important natural fiber crop cultivated in varying climatic conditions of tropics as well sub-tropic regions of more than 83 countries all over the world. Cotton plays a key role in the national economy in terms of generation of direct and indirect employment in the Agricultural and Industrial sectors. Due to ready availability of Bt-cotton seeds since 2002 and apparent advantages over non-Bt counterparts, its cultivation spread like wildfire in India within short span of time. Adoption of Bt cotton has not only changed the cultivation profile, but also the pest scenario. Changes in insect pest complex were evident with changed micro-climate. In the non-Bt era, the sprays applied for the bollworm control were able to keep sucking pest population under check. While there was a decline in the pest status of bollworms: the sap

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feeding insects emerged as serious pests (Vennila, 2008). This has happened due to vast cultivation of susceptible Bt cotton hybrids *(Gossypium hirsutum)* coupled with reduced application of insecticides. Among the sap sucking insects, loss caused due to leafhoppers *Amrasca biguttula biguttula* (Homoptera: Jassidae) also known as *Empoasca devastans* Dist., *Amarasca biguttula, Amrasca devastans* was of concern to farmers, researchers and policy makers. Besides cotton, it infests okra, brinjal, cacao, pepper, potato etc and is geographically distributed across Asia, Australia, and the Pacific Islands. Both nymphs and adults of leafhoppers suck the cell sap from the plant tissue and inject toxin into it resulting in 'hopperburn' symptoms. The affected leaves show crinkling and curling symptoms almost all over the plant and in extreme situations resulted in drying of leaves, reduced photosynthetic activity, that hampered the productivity of the cotton reducing average cotton yield upto 30% if not managed properly. About 25% yield losses in non Bt-cotton due to leafhoppers was estimated (Bhat *et al.*, 1994; Banerjee *et al.*, 2006) while yield losses were seen in Bt-cotton (Surulivelu *et al.*, 2009), indicated that leafhopper has been selected for more than one mechanism of resistance. In the recent study conducted (CICR, 2010) leafhoppers have developed resistance to some of the widely used insecticides like Imidacloprid, Thiomethoxam, Acephate, Monocrotophos up to 5040, 2500, 110 and 54 fold respectively in some pockets of India

Management practices of any pest are mainly based on the life cycle parameters of the insect to target susceptible stages before it reaches the economic threshold levels. A specific study related to the life cycle parameter of the leafhoppers on cotton is not clearly known especially under changing cropping systems. The developmental threshold of *A.b.biguttula* between lab and field conditions are varies, because the developmental duration may be influenced by abiotic factors such as variable temperature and humidity, food condition. An understanding of the life history of *E. devastans* is crucial to the integrated management of this pest. Successful management of the pest depends on the early detection and control of the leafhopper population. With high survival rates, rapid development, reproductive capacity, and lack of proper management, leafhopper populations can easily reach high levels and cause significant economic damage to cotton. For searching any alternative pest management strategy, it is imperative to study the population fluctuations through life cycle studies so that the weak link in the life cycle of the insect can be properly identified. The objective of this study was to determine the development of *E. devastans* on cotton during the season to obtain the information on vulnerable stages in life cycle that can be used for better management.

An experiment on life cycle parameters of *A.b.biguttula* was carried out on cotton leaves (*G. hirsutum*) at room temperature in the Insectary and Biocontrol Laboratory of Central Institute of Cotton Research (CICR), Nagpur during peak period of heavy infestation in the months of September to December 2010. Leafhopper affected leaves of cotton with slits on their veins were collected from cotton field to obtain newly emerged nymphs. The dust particles and other insects from the leaves

were removed and cleaned with water and kept in transparent plastic jar (500 ml capacity).

For rearing leafhopper, fresh tender leaves were collected from non infested cotton plants grown in cages, washed them under running tap water and dried. The petioles of the leaves were wrapped with water soaked cotton swab to keep cotton leaf fresh for a longer period. Out of the newly emerged nymphs, one day old 50 nymphs were transferred to fresh cotton leaves with soft camel hair brush and kept individually in the new plastic jar, secured with muslin cloth to prevent the escape of the nymphs. The desiccated leaves were replaced with fresh leaves daily and nymphs transferred individually on the fresh cotton leaves with soft hair brush.

The data on the developmental studies were recorded daily under stereo zoom microscope with photographic attachment (Leica). The moulting was confirmed by the presence of exuviae on the leaf or inner side of plastic jar. The colour and shape of each nymphal instar was observed. The individual nymphal period and instar number was recorded. The total nymphal period was then calculated from the period of egg hatching till the adult emergence. The newly formed adults were observed to study their colour, shape and sex difference.

For studying the fecundity data, 10 pairs of male and females of identical age were taken separately and kept in another plastic jar. Fresh cotton leaves were provided daily which also served for oviposition. Longevity of male and female was calculated separately right from the day of emergence till the death of adults. The adults were sexed based on the size and genital aperture (Tuxen, 1956).

Life cycle parameter studies of leafhopper, *A.b.biguttula* (Table 1) indicated that the first instar nymhal period ranged between 2–4 days, while next instars period recorded for second, third and fourth instar ranged from 1–5, 1–3 and 1–4 days, respectively. The average developmental period was found to be maximum in first instar (2.58 ± 0.57 days), followed by second instar (2.14 ± 0.81 days), third instar (2.03 ± 0.63 days) and fourth instar (1.84 ± 0.80 days). Newly hatched nymphs had transparent body with greenish yellow color and were very delicate (Plate 1). Eyes were oval in shape, reddish brown in color and look prominent. The second instar nymph was transparent with greenish yellow body, but slightly differing in morphology from first instar with regard to its eyes (Plate 2). It has white superficially dark reddish color underneath the eyes with rudimentary wing pads present all along the posterior margin of sides of pterothorax. The third instar nymph morphology was found almost similar to second instar in its appearance in except the color i.e. yellowish green in color (Plate 3). In this instar, wing pads developed prominently on pterothorax. Fourth instar nymphs were stout with dark yellowish green color and developed wing pads, which reached up to middle of abdomen (Plate 4). Eyes were more prominent and oval shaped.

The total nymphal period varied from 5–16 days with an average of 8.59 days which was less than the adult duration of 13.37 ± 5.17 days. Adult longevity was 37% more than nymphal period. In total 26% mortality was observed in nymphal development period till the emergence of adult. The adult life period was completed between 4 to

Nymphal stages

Plate 1. I[st] Instar

Plate2. II[nd] Instar

Plate 3. III[rd] Instar

Plate 4. IV[th] Instar

Adult abdomen

Plate 5. Female

Plate 6. Male

Adult

Plate 7. Full grown adult

PLATES 1–7 .

25 days with mean duration of 13.37 ± 5.17 days. The male life longevity was found between 15–34 days with mean of 21.38 ± 5.39 days. While the female life cycle was observed in the range of 17–37 days with an average of 23.19 ± 4.86 days indicting that female survive longer than males. Both male and female adults have prominent

TABLE 1. Life period of different developmental stages of *A.b.biguttula* under laboratory condition

Life stages	Life period (Days)*		Per cent
	Range	Mean ± SD	Mortality
I instar	2–4	2.58 ± 0.57	16.00
II instar	1–5	2.14 ± 0.81	7.14
III instar	1–3	2.03 ± 0.63	5.13
IV instar	1–4	1.84 ± 0.80	2.63
Total nymphal period	5–16	8.59	26.00
Adult longevity	4–25	13.37 ± 5.17	5.41
Male total life cycle	15–34	21.38 ± 5.39	–
Female total life cycle	17–37	23.19 ± 4.86	–
Male:Female ratio	1:1.31		

*N = 50

black spots on both sides of the median line in the vertex of the head and another in the apical area of the forewing. The nymphs and adult walk diagonally, face usually pale greenish, tegmina shining and wings hayaline iridescent. Fore wings were yellowish green in color.

Mortality was highest (16%) in first instar nymphs, followed by second instar (7.14%), adults (5.41%), third instar (5.13%) and fourth instar (2.63%). Regarding male genetelia, the sternum of the ninth segment was distinct, forming a semicircular or triangular plate, the genital valve. Behind the genital valve, the genital plates have two horizontal triangular plates that form the bottom of the genital capsule. Basically the aedeagus articulates by the atrial rim or the pre-atrium with the apex of the connective, a usually well sclerotized formation belonging to the phallobase. Laterally, the connective articulates with the movable styles or parameres, more or less elongate sclerotized processes and are placed on the dorsal side of the genital plates, with the apices directed backwards and more or less concealed by the plates (Plate 5).

In case of female, the tergum of the 9th abdominal segment forms pygofer, the lateral pairs of which are the valvulae laterals. In a ventral groove of the pygofer lies ovipositor. The latter consists of three pairs of oblong valves *viz.*, one pair emerging from the 8th and two pairs from the 9th abdominal segment. They are basally attached to so called valvifers and these pairs rest on the 8th and 9th abdominal sterna. The posterior pairs of valves (lateral valves, third valvulae) of the 9th sternum encloses laterally, the remaining two pairs of valves (the saw) and is termed as the saw-case. The saw consists of two pairs of generally more or less saber-like sclerites, one outer pair belonging to the 8th sternum (anterior valves, first valvulae) and one inner pair (median/inner valves, second valvulae), arising from the anterior part of the valvifers of the 9th abdominal segment (second valvifers) (Plate 6). At the end of the life cycle a total of 37 adult survived, out of which, 21 were female and 16 males indicative of higher number of females than males with sex ratio biased towards females (1:1.31 male:female). The egg stages could not be located and were difficult to identify under

the plant tissues as the pair of male and female adults placed on fresh cotton leaves did not lead to eggs or neonates.

The developmental threshold of most of the insects between lab and field conditions tend to vary owing to environmental and food factors. An understanding of weak link in the life history of insect is very crucial to the integrated management of this pest. In the present investigation leafhoppers complete nymphal developmental periods 2.58 ± 0.57, 2.14 ± 0.81, 2.03 ± 0.63 and 1.84 ± 0.80 days in first, second, third and fourth instars, respectively. The first and second instar life periods are in agreement with the reports of Jayaraj and Basheer (1964) and Shivanna *et al.* (2009). The results of Sharma and Sharma (1997) and Parmar *et al.* (2006) regarding third instar duration are similar with present investigation. The results of morphology of leafhopper and fourth instar nymphal period are close with the findings of Shivanna *et al.* (2009). In the present study, total nymphal period varied from 5–16 days with an average of 8.59 days which is less than the adult period of 13.37 days. In earlier studies, life cycle of leafhopper was completed in less than 30 days on cotton (Huque, 1994). Our results are comparable with the findings of Sharma and Sharma (1997) who observed 7.30 days as nymphal period of leafhoppers in their study. However, longer nymphal period (11.68 days) was found by Shivanna *et al.* (2009).

Adults have prominent black spots on both sides of the median line in the vertex of the head and another on the apical area of the forewing (Plate 7). The nymph and adult walk diagonally, face usually pale greenish, tegmina shining and wings hayaline iridescent. Forewings were yellowish green in colour. Similar types of observations were recorded by Vashi (1968) and Shivanna *et al.* (2009) with respect to morphology and duration.

When kept on fresh leaves with a pair of adults, the eggs could not be located in the present study. But, earlier reports regarding the egg laying habit, Sharma and Singh (2002) observed eggs of *E. biguttula biguttula* on lateral veins of cotton leaves. While, Shivanna *et al.* (2009) reported that the *E. biguttula biguttula* female inserted the eggs into plant tissues such as tender petioles, twigs and succulent leaves, but mainly on midrib and veins of under surface of leaves and majority of eggs were laid in midrib tissues. The observations were started from the newly emerged nymphs, which had transparent body with green yellowish in color and were very delicate. Parmar *et al.* (2006) and Shivanna *et al.* (2009) reported similar findings. In present study, the nymph of *E. devastans* passed through four instars. We report four nymphal instars while five nymphal instars were reported by Jayaraj and Basheer (1964) in *Empoasca flavescens* (Fab.) and Shivanna *et al.* (2009) in *E. biguttula biguttula*. Studies on bionomics of E. *devastans* was carried out by on some varieties of cotton at Lyallpur in 1935–37 (Husain and Lal, 1940), however they have not provided such elaborative observations.

The present study on life cycle parameters of cotton leafhopper, *E. devastans* on cotton revealed that the early two instars were found more vulnerable to death than later and adult, indicated emphasizing more stress on early nymphal stages

when deciding management strategies. In this regard, current study will be helpful in deciding the life period of leafhopper after initiation of incidence under field situation.

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Cytopathological and enzymatic changes in the hemolymph of *Oryctes rhinoceros* grubs in presence of various stressors

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ABSTRACT: Exposure of *Oryctes rhinoceros* grubs to various physical, chemical and biological stressors have resulted in an elevation of lactic acid (LA) together with an increase in NADH dependent - Lactate Dehydrogenase [LDH] activity but the enzymes concerned with glycolytic series such as Hexokinase and Glycogen Phosphorylase showed a sharp decline of activity. Even though the mitochondrial enzymes Malate Dehydrogenase and Succinate Dehydrogenase became very low in their activity under all the stress conditions, another mitochondrial enzyme Glutamate Dehydrogenase showed sharp elevation, possibly through the coupling of Kreb's cycle and transamination process. Native PAGE (nPAGE) of hemolymph and subsequent localization of SDH activity has revealed that the grubs possessed three isozymes and all the three were active only in normal life. Under various types of experimental stress conditions one or two of the isozymes bands disappeared which indicated that insects have adopted transcriptional and/or translational control of some genes to overcome the stress. Under all the stress conditions a sharp reduction in the Total Hemocyte Count (THC) and a drastic numerical change in the different population of hemocytes with a selective increase of granulocytes (almost 45%) were observed. This indicated the important role of granulocyte in eliminating alien cells, alien protein and live bacteria, and also in helping the organism to adjust with the suddenly altered temperature. Confocal Laser Scanning Microscopic studies revealed the disintegration of nuclear membrane and mitochondria of hemocytes on exposure to various stressors.

KEYWORDS: *Oryctes rhinoceros*, Lactic Acid, Succinate Dehydrogenase, THC, DHC, CLSM.

INTRODUCTION

Insects occupy a wide range of ecologically diverse niches and hence, they exhibit a variety of strategies to overcome various types of stressors while as developing

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state or as adult. Insects can drastically decrease their metabolic rate in response to various environmental and physiological conditions such as exposure to varying concentrations of CO_2 (Guo *et al.*, 2013), starvation (Harshmann *et al.*, 1999) and at low temperature (Singh *et al.*, 2010). On exposure of the insects to stress, gene expression of various respiratory and intermediary metabolic enzymes are either up regulated or down regulated, and stress tolerance mechanism is enhanced to protect cell attributes and enzymes required for resumption of growth and development. Trehalose, a major disaccharide in insects has been reported to act as an anti freeze agent, protecting the cells from potent cell damage and maintains membrane fluidity, on exposure of the insects to various temperature stress. In order to tolerate various stressors, specific changes occurs from one type of intermediary metabolism to another together with a depression in their metabolic pathways as well as diversion of energy expenditure away from cellular events. Majority of insects avoid stress exigencies through, a dynamic process, diapause. Hyperproteinaemia of the hemolymph was observed in adults and larval insects on exposure to various stress conditions (Adhira *et al.*, 2010). Thus insects adopt effective and well organized strategies such as shift in energy metabolism for survival in extreme stress conditions, which form the subject matter of this communication.

MATERIALS AND METHODS

Insects

Healthy and actively feeding 3rd instar larvae of *Oryctes rhinoceros* collected from various compost and cow dung pits from in and around Trivandrum, Kerala were reared in plastic containers (11 cm X 8 cm) containing sterile cow dung, under laboratory conditions at an ambient temperature of 27 ± 2°C (Sreekumar and Prabhu, 1988). Larvae having an average weight of 11.6 ± 1.02 g were selected for the study.

Exposure of insect larvae to various stressors

Intrahemocoelic Challenge of with Bacteria, Alien proteins and Chick RBCs.

O.rhinoceros grubs were challenged with intrahemocoelic injection of live bacteria such as an entomopathogenic *Bacillus thuringiensis israelensis*, a non-entomopathogenic *Escherichia coli*, alien proteins like Ovalbumin and Casein and alien cells such as chick RBCs. The site of injection was cleaned with 70% ethanol prior to all intrahemocoelic administration. Live bacteria were administered into the larval body by intrahemocoelic injection, using a sterile insulin syringe, at a dose containing 10^4 bacteria/larvae. Ovalbumin and casein dissolved in phosphate buffered saline (PBS) (pH: 7.2) were administered into the body of the larvae by intrahemocoelic injection using a sterile insulin syringe at a dose of 30 micrograms/larvae. Chick RBCs washed and suspended in PBS were injected into the larval hemocoel at a dose of 4000 cells/larvae.

In all the above intrahemocoelic challenges, the hemolymph from the larval body was withdrawn for the study after twelve hours. Intrahemocoelic challenges were

done after proper dose response study with at least 6 doses and in all such injections the volume was limited to 30μl. Twelve larvae were taken for each intrahemocoelic injections. A group of ten larvae, taken as the control, were injected with equal amounts of PBS.

Exposure to Thermal Stress

Larvae reared in sterile cow dung at 27 ± 2°C, were kept in experimental cages that were maintained at 5°C and 47°C for a period of three hours. Hemolymph was withdrawn after three hours. Twelve larvae were taken for each temperature treatments. A group of ten larvae were taken as control.

Mite Infection

A parasitic mite, *Poecilochirus sp.*, found on the body surface of *Oryctes rhinoceros* larvae were carefully separated from the larval body by using a smooth painting brush with minimum disturbance. These mites were transferred into fresh sterile cow dung at a density of 50 mites/100 gm cow dung. Six healthy larvae were released into the mite infested cow dung of 100 gm. Hemolymph was withdrawn for the study after five days of infection.

Hemolymph and hemocyte collection

Hemolymph from both the normal and infected larvae was collected, by cutting its 3rd prothoracic leg and gently squeezing its body and stored in sterile Eppendorf tubes in a deep freezer. Biochemical studies were carried on the cell free hemolymph after centrifugation at 14,000 rpm at 4°C for 15 mins.

Enzyme assays

Quantitative estimation of Lactic Acid (LA) (Baker, 1940), NADH-Dependent Lactate Dehydrogenase (LDH) [E.C. 1.1.1.27] (Queen, 1972), Hexokinase [E.C. 2.7.1.1] (Brandstrup *et al.*, 1957), Glycogen Phosphorylase (GP) [E.C. 2.4.1.1] (Singh *et al.*, 1961), Succinate Dehydrogenase (SDH) [E.C. 1.3.5.1] (Pennington, 1961), Malate Dehydrogenase (MDH) [E.C.1.1.1.37] (Mehler, 1948), and Glutamate Dehydrogenase (GDH) [E.C. 1.4.1.2] (Strecker, 1955) was carried out using standard protocols.

Localisation of enzyme activity in native gel

The qualitative analysis of SDH activity was carried out in Native Poly Acrylamide Gel Electrophoresis (nPAGE) with the discontinuous buffer system containing 5% stacking and 8% separating gel. The gels, soon after the removal, were washed in distilled water followed by the incubation in 100 ml of sodium phosphate buffer (50mM pH 7.0) containing sodium EDTA 400 mg, sodium succinate 250 mg, ATP-Na_2 50mg, NAD^+ 70 mg, NBT (Nitro Blue Tetrazolium) 40 mg and phenazine methosulfate (PMS) 2mg at 37°C in rotary shaker in dark for 30mins. The gel was scanned and photographed in a gel scanner (GEL SCAN), and analyzed using LABIMAGE PLATFORM software.

Cytopathological and hematological studies

Hematological studies such as Total Hemocyte Count [THC] and Differential Hemocyte Count [DHC] were carried out on the same day of sacrifice. THC and DHC were carried out according to the method devised by Jones, 1962, using a powerful Leica microscope. The percentage of different types of hemocytes in each sample was calculated for DHC. Nuclear fragmentation and changes in the mitochondria have been analyzed through Confocal Laser Scanning Microscopic [CLSM] studies using fluorescent dyes such as DAPI and Mito Tracker Green.

Statistical analysis of data

The data obtained are represented as Mean ± Standard Deviation. Statistical comparisons were performed by one way analysis of variance [ANOVA] followed by Duncan's multiple range test [DMRT] using SPSS 21 software Daniel (2006). The results were considered statistically significant if $p \leqslant 0.05$.

OBSERVATION AND RESULTS

Intrahemocoelic injection of *O. rhinoceros* larvae with live bacteria, *Bti* and *E.coli* has resulted in a sharp elevation of hemolymph lactic acid (LA) which was thrice the normal value at the 12th hour. Exposure of grubs to 5°C and 47°C for three hours has caused a double fold elevation of LA which was equivalent to the LA content in the larvae which was experimentally infected with the ectoparasitic mite, *Poecilochirus sp* for five days. Intrahemocoelic injection of alien cells such as cRBCs caused a three fold elevation of LA, but alien proteins such as ovalbumin and casein caused only 25% and 50% elevation respectively and results will be clear from Fig. 1.

In all the above mentioned stress conditions elevation of LA showed a positive correlation with the activity of NADH dependent Lactate Dehydrogenase (LDH). Exposure of the larvae in 5°C and 47°C for three hours was sufficient for a threefold elevation of LDH activity; but experimental attack by *Bti* and *E.coli* has caused a fourfold increase of LDH activity. Compared with the effect of Ovalbumin and Casein on LDH activity, the effect of avian RBC on LDH was more (Fig. 1).

A major enzyme, Hexokinase, which is the first one in the path of glycolytic series, showed a sharp decline in the activity under all the above mentioned stress condition (Table 1). Another carbohydrate metabolizing enzyme, Glycogen Phosphorylase (GP) has also undergone a reduction in its activity under all the experimental stressors. The stressors such as low temperature (5°C) and *Bti* caused the activity reduction of GP to one-fifth of the normal range.

The mitochondrial enzymes, Succinate Dehydrogenase and Malate Dehydrogenase showed depletion in its activity in the presence of all the above mentioned stress conditions (Fig. 2). Exposure of the larvae to 5°C and 47°C for a period of three hours was sufficient for the drastic reduction in its activity of SDH and MDH to one-third and half of the original activity respectively. Intrahemocoelic challenge by *E.coli* and *Bti* caused the activity of the above two enzymes to undergo reduction to one fifth

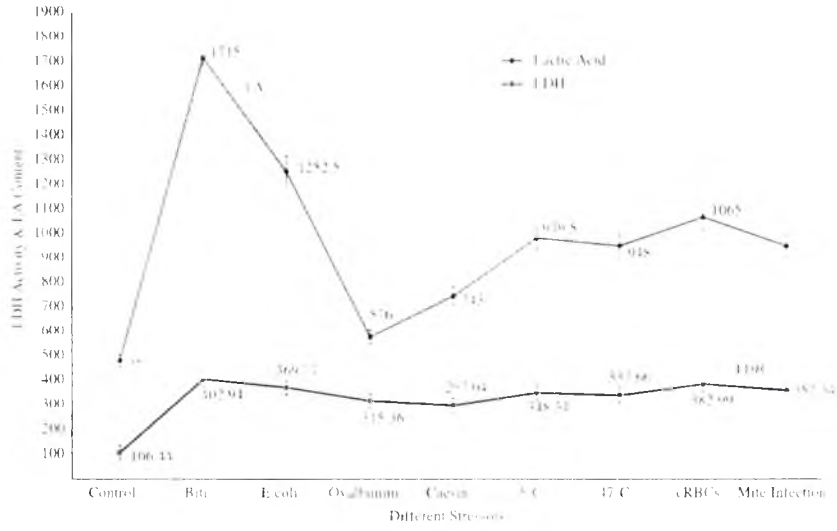

FIGURE 1. Latic acid levels and LDH activity in the Hemolymph of *Oryctes Rhinoceros* Grubs in response to stressors.

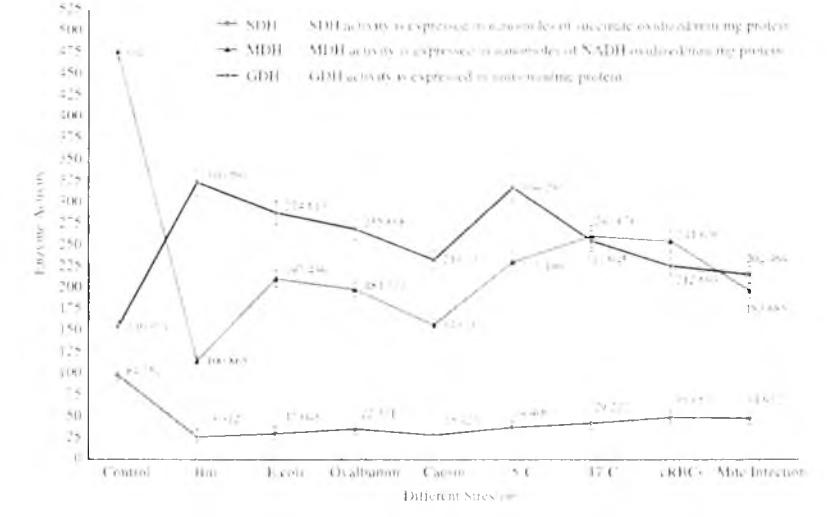

FIGURE 2. Enzymatic profiles of certain Mitochondrial enzymes in the Hemolymph of *Oryctes Rhinoceros* Grubs in response to stressors.

TABLE 1. Enzymatic profile of Hexokinase and Glycogen Phosphorylase in the Hemolymph of *Oryctes rhinoceros* Grubs on eposure to stressors.

Different Stressors	Hexokinase[#]	Glycogen Phosphorylase[##]
Control	124.349 ± 7.81	320.384 ± 27.47
Bti	23.561 ± 1.76	53.522 ± 4.03
E.coli	41.413 ± 3.57	56.347 ± 4.82
Ovalbumin	67.090 ± 5.69	79.297 ± 7.31
Casein	62.027 ± 5.51	71.843 ± 6.58
5°C	73.221 ± 6.85	67.518 ± 6.11
47°C	75.736 ± 7.12	107.739 ± 10.14
cRBCs	82.937 ± 7.96	95.653 ± 8.86
Mite Infection	87.008 ± 8.04	84.508 ± 8.05

(All values are Mean ± Standard Deviation. All values are significant at $p \leq 0.05$)

of its original value. On the other hand, Glutamate dehydrogenase (GDH) showed a double fold elevation in its activity under all the stress conditions (Fig. 2).

Localization of SDH isozymes by native PAGE showed the presence of three isozymes for SDH. The zymogram also revealed a difference in the thickness of certain bands as well as the absence or disappearance of certain bands during the course of exposure to various experimental stressors (PLATE I).

The Total Hemocyte Count (THC) showed a sharp decline in the case of larvae exposed to 47°C and 5°C as well as in the larvae administered with intrahemocoelic injections of alien proteins, live bacteria and cRBCs. The THC in the case of mite infected larvae also showed depletion in its count. Results are shown in Fig. 3.

Differential Hemocyte Count (DHC) showed a significant increase in the number of Granulocytes after exposure of the larvae to stress, while in the normal larvae, Adipohemocytes were found to be abundant. DHC of both the normal and stress exposed larvae on percentage basis and are shown in Fig. 4. Six different types of hemocytes have been identified in the normal larvae (Jones, 1963), viz. Prohemocytes, Plasmatocytes, Adipohemocytes, Granulocytes, Oenocytoids and Spherulocytes. Confocal Images shows nuclear degradation in hemocytes of larvae exposed to various stressors, indicating lack of integrity of nuclear membrane (PLATE II). Even though not much damage to the mitochondria has been observed on exposure of the grubs to various physico-chemical and biological stressors, some of them appeared diffused (PLATE III).

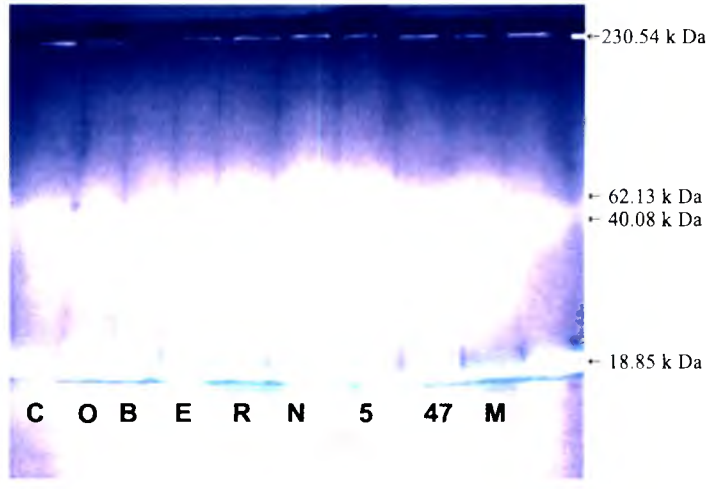

PLATE I. Native Page Electro phorogram showing the localisation of Different SDH Isosymes of *Oryctes rhinoceros* grubs on exposure to various stressors

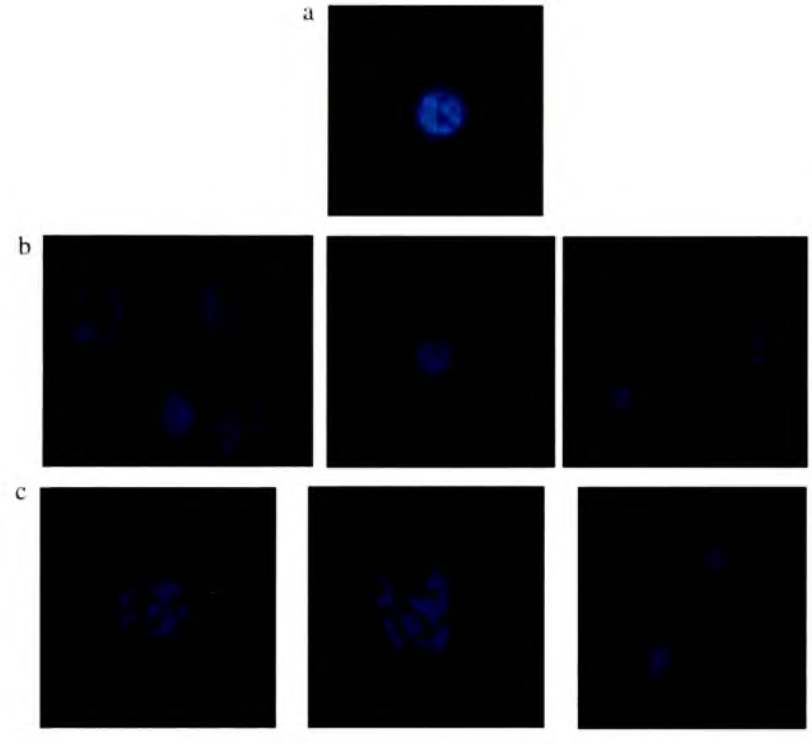

PLATE II. Confocal laser scanning images of Nuclear damage observed among hemocytes of the larvae on exposure to various stressors.
a - Normal nucleus; b - c - various stages of nucleus disintegration in the hemocytes

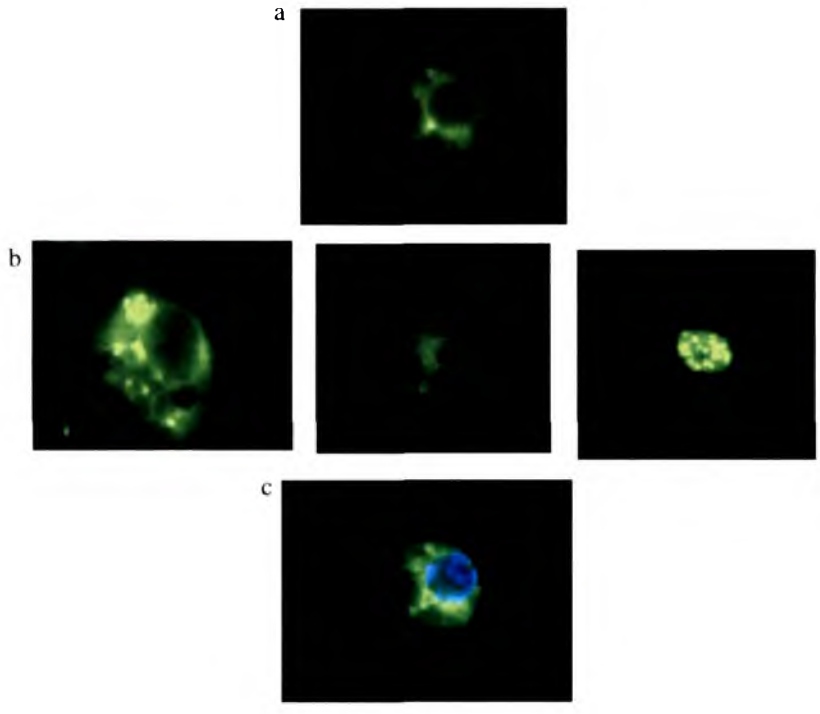

PLATE III. Confocal laser scanning images of mitochondrial damage observed among hemocytes on exposure to various stressors:
a. Normal mitochondria, b. Mitochondrial damage visualised in hemocytes on exposure to stressors, c.Merged image.

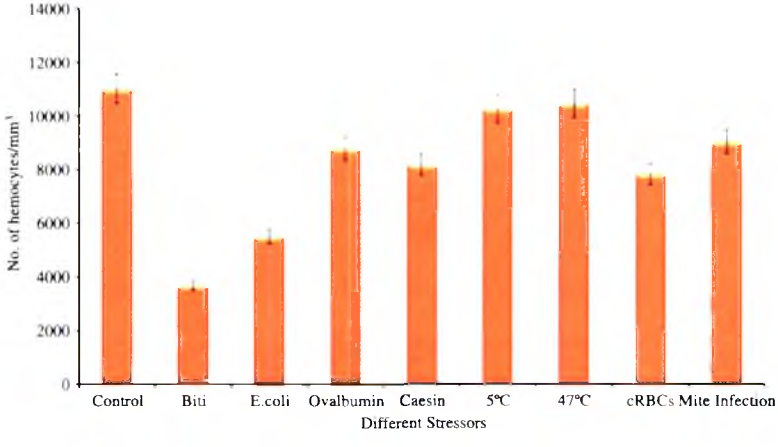

FIGURE 3. Total Hemocyte count of *Oryctes rhinoceros* larvae exposed to various Stressors.

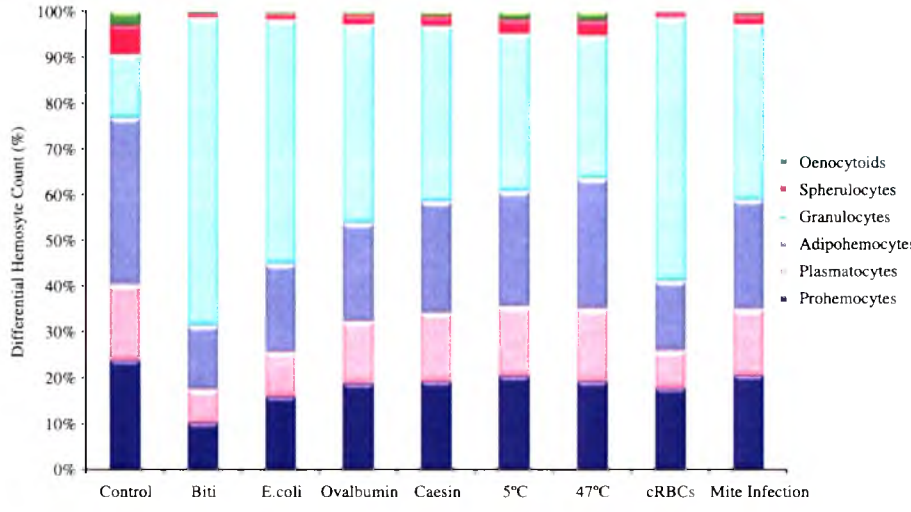

FIGURE 4. Differential Hemocyte count of *Oryctes rhinoceros* larvae exposed to various stressors.

DISCUSSION

Oryctes rhinoceros grubs when subjected to various physical, biological and chemical stressors resulted in the accumulation of LA, which can be correlated to the increase in the activity of LDH. Accumulation of LA in the hemolymph of insects such as honey bees on exposure to stressful environments has been reported by other investigators (Shamitha and Purushotham, 2008; Denlinger and Richard, 2010). Enhanced LDH activity, in the present study, indicated the inhibition of lactate conversion to pyruvate, resulting in a shift from aerobic to anaerobic metabolism for meeting the required energy demands under stress conditions (Hamadah *et al.*, 2010).

Decreased activity of Hexokinase and GP are indicative of the chance that the larvae may be entering diapauses by decreasing its metabolic rate. Similar decrease in these house-keeping enzymes has been reported in *Drosophila melanogaster* on exposure to phytochemicals as well as on starvation (Wang and Clark, 1995) (Hoffman and Parsons, 1991, Harshmann *et al.* 1999)

Suppression in the activities of mitochondrial enzyme, SDH and MDH under various stress conditions could be due to an overall reduction in number of functional mitochondria, reduced amounts of enzyme proteins per mitochondrion, or suppression of enzyme activity via post-translational regulation. Similar observations have been reported in insects exposed to cold stress (Joanisse Storey, 1994).

Elevation of GDH activity among the larvae on exposure to various stressors clearly indicated the coupling of GDH and Transaminase (AsAT and AlAT) enzyme in the normal grubs as well as those exposed to stress conditions. Increased transamination of amino acids and onset of detoxification mechanism to overcome the stress conditions

was observed in this organism and has already been reported (Adhira, 2013). It also indicates impairment in nitrogen metabolism. Similar observations have been documented in silkworm and pea aphids on exposure to pesticidal and environmental stressors (Nath *et al.* 1997) (Guo *et al.*, 2013).

The Total hemocyte count (THC) of larvae exposed to various stressors showed a significant decrease compared to control. Various cytopathological changes such as distortion of cell shape, abnormal staining pattern, cell enlargement, denucleation and rupturing of the cell membrane were observed mainly among the Plasmatocytes of the larvae (Adhira *et al.*, 2010). Plasmatocytes and granulocytes are the phagocytic cells, where the former phagocytose non-self cells and the latter phagocytose dead cells (Eleftherianos *et al.*, 2009; Strand, 2008). Similar results were reported by various investigators in other insects in response to stressors (Pandey *et al.*, 2008; Turnbull *et al.*, 2004; Tiwari and Shukla, 2000). The differential hemocyte count (DHC) revealed that even though the THC showed numerical decrease, the population of granulocytes (GRs) showed a sharp elevation, up to 45–50% than the normal, when exposed to various stressors. Similar results were obtained in our preliminary studies when the larvae were fed with cow dung contaminated with *Bti* spores (Adhira *et al.*, 2010). Similar results have been reported in other insects (Barbara *et al.*, 2011). This indicated that granulocytes play a very important role in defense against pathogens, foreign proteins, antigens, in parasitic infection as well as in response to exposure to cold and hot temperature stresses through cell mediated and humoral mediated immunity, which requires a detailed study. Confocal Laser Scanning Microscopic studies also show various degrees of nuclear disintegration; this could strongly indicate the possibihty of DNA damage, which needs further studies in detail.

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Observations on the life history of two species of predatory mites inhabiting medicinal plants of Kolkata

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ABSTRACT: The present study deals with the different developmental stages of life cycle along with fecundity of two important predatory mites namely, *Pronematus sextoni* Baker on *Bauhinia acuminate* Linn. and *Euseius ovalis* (Evans) on *Justicia adhatoda* Linn. under laboratory condition. Duration of egg to adult stage of *P. sextoni* was noticed as 6.0 ± 0.25 days, whereas in case of *E. ovalis* it was 4.16 ± 0.47 days only. Fecundity of *P. sextoni* was 9.15 ± 0.32 per day per female. On the other hand, fecundity of *E. ovalis* was calculated 1.27 ± 0.09 eggs per day per female, indicating species-specific variations in the life history parameters.

KEYWORDS: predatory mites, medicinal plants, developmental stages, fecundity

INTRODUCTION

Predatory mites are used as potential bio-control agent for successful control of phytophagous mites and thus gaining worldwide importance (Gerson *et al.*, 2003). In recent past, several new reports of predatory mites infesting medicinal plants in West Bengal, India were published (Roy *et al.*, 2006, 2008a,b, 2009, 2010; Roy and Saha, 2010). However, practically no study has been made in India on the biology of the predatory mites on medicinal plants. The present paper deals with the life cycle (the duration of different developmental stages including egg, larva, nymph and adult) of two selected major species of predatory mites based on the abundance in the respective host plants, both at the geographical and temporal scale. The life cycle study of predatory mite *Pronematus sextoni* Baker was carried out on *Bauhinia acuminate* Linn. at a mean temperature and relative humidity of 26°C and 75 percent respectively, during July 2006 and life cycle of another predatory mite *Euseius ovalis* (Evans) was

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recorded on *Justicia adhatoda* Linn. under laboratory condition at a mean temperature and relative humidity of 22°C and 70 percent respectively, during November 2005.

MATERIALS AND METHODS

In the laboratory, adult mites (collected time-to-time from Experimental Garden at Ballygunge Science College Campus, Kolkata and Medicinal Plants Garden, Narendrapur Ramakrishna Ashrama, Narendrapur), were cultured on the freshly plucked leaves kept on a wet cotton pad in plastic trays measuring 30 × 25 × 6 cm in the laboratory at 30 ± 0.33 °C and 77.45 ± 1.06% RH inside the BOD incubator.

To study the life cycle and features of developmental stages the naturally occurring adult females were introduced on leaf (1 per Petri dish and 20 sets) placed on a wet cotton pad in a Petri dish (Tarsons) measuring 8.5 cm diameters and 1.3 cm in height. Different life stages including eggs of *Tetranychus ludeni* (from mass culture at laboratory) were used as source of food. The cotton bed was kept wet by soaking with water twice daily so that the discs remained fresh. The mites were allowed to lay eggs. After hatching, the larvae were transferred carefully to a prepared leaf-disc at the rate of one larva/leaf disc/Petri dish. Precautions were taken to remove all organisms on the leaves by brushing and examining the leaf under stereo-binocular microscope (Gupta *et al*., 2003; Ghoshal *et al*., 2004).

The duration of each life stage were recorded at 12-hour intervals until they reached adulthood. The data thus collected were subjected to calculate mean duration of different developmental stages. To measure fecundity, the number of eggs laid was counted daily until the female died.

RESULTS

Life cycle of predatory mite *Pronematus sextoni* was studied on *Bauhinia acuminate* Linn. under laboratory condition at a mean temperature and relative humidity of 26°C and 75 percent respectively, during July 2006. The life cycle included egg, one larval stage, two nymphal stage and adult stage. The duration of individual life stages are depicted in Table 1. The life cycle study of another predatory mite *Euseius ovalis* was observed on *Justicia adhatoda* Linn. under laboratory condition at a mean temperature and relative humidity of 22°C and 70 percent respectively, during November 2005. The fecundity and the duration of individual stages are presented in Table 2.

Eggs

The eggs of *Pronematus sextoni* are elliptical and transparent measuring about 78 μ. The mean incubation period of eggs was 1.2 ± 0.34 days with minimum of 1 day and maximum of 2 days.

In case of *Euseius ovalis*, the eggs were spherical and transparent. Minimum of 1 day and maximum of 3 days with mean incubation period of 2.0 ± 0.19 days were recorded.

TABLE 1. Duration of different developmental stages and fecundity of *Pronematus sextoni* Baker on *Bauhinia acuminate* Linn. under laboratory condition

Stage	Range (in days)	Duration ± SE (in days)
Egg	1–2	1.2 ± 0.34
Larva	2–3	2.3 ± 0.31
Protonymph	2–3	2.5 ± 0.32
Deutonymph	2–3	2.5 ± 0.32
Egg to adult	5–10	6.0 ± 0.25
Adult longevity	14–20	18.16 ± 0.48
Fecundity (Day^{-1} $Female^{-1}$)	7–14 eggs	9.15 ± 0.32 eggs

For durations, the values given are mean of twenty replications.
For fecundity, the values given are of five replications.

Larva

The duration of mean larval period of *P. sextoni* was 2.3 ± 0.31 days. Minimum of 2 days and maximum of 3 days were noticed.

In case of *E. ovalis*, the duration of mean larval period was of 1.35 ± 0.48 days.

Protonymph and deutonymph

The duration of mean nymphal stage of *P. sextoni* was 2.5 ± 0.32 days with minimum of 2 days and maximum of 3 days observed in both protonymph and deutonymph.

In case of *E. ovalis*, the mean duration of protonymph and deutonymph were recorded as 1.1 ± 0.30 days and 1.16 ± 0.11 days with minimum of 1 day and maximum of 2 days.

Adult longevity

Adult longevity of female *P. sextoni* was 18.16 ± 0.48 days recorded, but adult (female) longevity was 9.66 ± 0.41 days observed in *E. ovalis*.

Egg to adult

Duration of egg to adult stage of *P. sextoni* was 6.0 ± 0.25 days noticed whereas in *E. ovalis* it was 4.16 ± 0.47 days only.

Fecundity

Fecundity of *P. sextoni* was 9.15 ± 0.32 per day per female. On the other hand, fecundity of *E. ovalis* was calculated 1.27 ± 0.09 eggs per day per female.

TABLE 2. Different developmental stages and fecundity of *Euseius ovalis* (Evans) *Justicia adhatoda* Linn. under laboratory condition

Stage	Range (in days)	Duration ± SE (in days)
Egg	1–3	2.0 ± 0.19
Larva	1–2	1.35 ± 0.48
Protonymph	1–2	1.1 ± 0.30
Deutonymph	1–2	1.16 ± 0.11
Egg to adult	4–5	4.16 ± 0.47
Adult longevity	8–11	9.66 ± 0.41
Fecundity (Day^{-1} $Female^{-1}$)	1–2 eggs	1.27 ± 0.09 eggs

For durations, the values given are mean of twenty replications.
For fecundity, the values given are of five replications.

DISCUSSION

Practically no study from India was made on biology of Tydeidae family. Recently Roy *et al.* (2009) studied the biology of *Tydeus justicia* Roy *et al.* on *Justicia adhatoda*, a common plant of medicinal importance. It is apparent that the duration of egg to adult period of *Pronematus sextoni* (6 ± 0.25 days) takes almost similar time as that of *Tydeus justicia* (7.67 ± 0.47 days). Duration of egg to adult stage of *Euseius ovalis* on *Justicia adhatoda* Linn. was 4.16 where as it was 5.29 days as studied by Borah and Rai (1989). Duration of total developmental period of predatory mite species *Amblyseius* (*Euseius*) *concordis* was 66.80 ± 1.14 hrs. at 35 ± 1°C temperature with 55 ± 3 percent relative humidity and 120.48 hrs. at 20 ± 1°C temperature with 60± 1 percent relative humidity, respectively (Jagadish *et al.*, 1995). Saha *et al.* (1988) studied that the total developmental period of another predatory mite *Amblyseius cocosocius* was 4.83 ± 0.22 days at 35 °C temperature and 8.53 ± 0.17 days at 30 °C temperature, respectively. Average fecundity of *E. ovalis* appeared to be very poor that was only 1.27 ± 0.09 eggs per day per female whereas *Amblyseius channabasavannai* laid 2.7 ± 0.60 eggs per day per female (Daniel, 1981). Other species of *Amblyseius* showed higher fecundity rate as evident from reports of Jagadish and Nageshchandra (1982), Sharma and Sadana (1985, 1986); Gupta *et al.* (2003).

The life cycle features of *Pronematus sextoni* and *Euseius ovalis* studied under laboratory conditions, indicated species specific variations in the life history parameters like duration of instar stages, longevity, and fecundity. The lifecycle of *P. sextoni* has been evaluated for the first instance in India. Considerable variation in the life cycle data of *E. ovalis* were noted contrast to different geographical situations reported. Thus the life cycle data of these mite species will help to compare and predict possible abundance pattern in nature.

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Biology and rearing performance of *Cricula trifenestrata* Helfer (Lepidoptera: Saturniidae), a wild silk moth in Nagaland, India

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ABSTRACT: *Cricula trifenstrata* Helfer is bivoltine in nature and undergoes seven months pupal hibernation in Nagaland climatic condition. The worm is polyphagous in nature and feeding on three host plants out of which detail rearing was conducted in two host plants, namely *Persea bombycina* and *Litsea citrata*. The total larval duration of *C. trifenestrata* was shorter in *P. bombycina* (40.70 ± 1.56 and 48.50 ± 1.02 days) in comparison to *L. citrata* (46.30 ± 1.72 and 53.20 ± 0.97 days) in summer and autumn season respectively exhibiting significant influence of seasons and host plants on life cycle. While the yellow and mesh-like perforated cocoons exhibited colour morphism, no significant difference was observed between the seasons and host plants except for green cocoon weight and shell ratio of male. The important production parameters, i.e. fecundity (number), hatching percentage, effective rate of rearing (ERR) and cocoons per disease free laying was found to be higher on *P. bombycina* than *L. citrata* with no significant difference between the seasons. *C. trifenestrata* was found to be free from other silk worm diseases like pebrine, muscardine and infestation of uzi fly, ant, and wasp. Since both the host plants are grown abundantly in Nagaland, evaluation of suitable conservation and utilization strategies are required for establishment of this species as resourceful, disease resistant material towards flourishing the sericulture industry in the state.

KEYWORDS: *Cricula trifenestrata*, Wild silkmoth, Biology, Rearing performance, Nagaland, India

INTRODUCTION

Cricula trifenestrata Helfer is considered as one of the most destructive pests of mango damaging 13 to 51% of leaves in Bangladesh (Ahmad and Alam, 1994). It also infested Daruchini plant *Cinnamonum zeylanicum* and caused almost complete defoliation

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(Ahmad and Ahmad, 1991). In India the larvae of this species have been reported to feed on plantation crop (Das *et al*., 1999), cardamom (Yadav and Kumar, 2003) and cashew *Anacardium occidentale* (Pal and Medda, 2006). The larvae were seen feeding voraciously on leaves, resulting complete defoliation with only the mid-ribs being left. While *Cricula trifenestrata* is enlisted as one of the wild silk moths due to its natural silk producing characteristic, it is so far considered as the serious pest of laurel, *Persea bombycina* (King & Hook) Kosterm, the primary host plant of *Antheraea assamensis* in North-Eastern India. The worm has been found to feed on three host plants, namely, *Persea bombycina, Litsea citrata* Blume and *Betula alnoides* Buch-Ham ex D.Don in Nagaland (Kakati and Chutia, 2009). While silk producing character is not evaluated in India, the yellow and mesh-like perforated cocoons are used in many purposes in Indonesia (Singh and Suryanarayan, 2003). Indonesian Wild Silk moths Development Project totally changed people's perception of this worm which was long regarded as a useless insect and now many people enjoyed the beauty and advantages of its silk in Indonesia (Nurmalitasari and Kuroda, 2002). Further, except for preliminary study on morphometric measurements (Rono *et al*., 2008), no study on rearing and commercial utilization of this species has so far been reported. Hence a detail study was conducted on biology and rearing performance of *C. trifenestrata* on its two host plants i.e. *P. bombycina* and *L. citrata* in Mokokchung, Nagaland.

MATERIAL AND METHODS

Cocoons of *Cricula trifenestrata* were collected from *Litsea citrata* (Mejankari) plant in Mokokchung district in the month of July–August, 2005. In the same season some hairy caterpillars in their 5th stage were also observed on *Persea bombycina* (Som) in nearby forest area and allowed to spin to form cocoons. The cocoons were collected and kept in wooden cage until their emergence. The emerged moths were kept in moth coupling cage for pairing and there after the fertile females were tied in khorikas (bend wooden stick) for egg laying. After egg laying, the females were tested for disease freeness and the disease free layings along with khorikas were kept for hatching at room temperature of 17°C–19°C and relative humidity 78–84%. Equal numbers of newly hatched larvae were brushed on the selected bush plantations of *P. bombycina* and *L. citrata* (in triplicate) which were covered with nylon nets to protect silkworms from predators and other enemies. On maturity, the larvae were hand picked and kept in mountage for spinning of cocoons. Cocoons were harvested after 8–10 days of pupation. The rearing and grainage experiment was conducted with strict observation for four consecutive generations during 2005–2006. The data were recorded for study of biology which included the parameters such as morphometric characters (length, breadth and weight), incubation period, larval duration, adult longevity and fecundity taking ten replicates for each treatment. The abiotic factors were also recorded during the study period. The mean, standard deviation (SD) and standard error (SE) were calculated from the computed values. Randomized block design and completely randomized block design were used to analyse the field and laboratory data respectively (Gomez and Gomez, 1978). Further, critical differences

were calculated by using F-test as described by Snedechor and Cochran (1979). The measurement and commercial characters of cocoons was carried out by conventional method.

RESULTS AND DISCUSSION

Description of immature stages

The following descriptions were made from live specimens reared in two host plants i.e. *Persea bombycina* and *Litsea citrata*. The first instar was described with in 2 days after eclosion, while all other instars were described within 2–3 days following a moult. The measurements of various developmental stages are given in Table 1

Egg

Egg is ivory white in colour and round in shape. It measures 0.18 cm in length, 0.12 cm in breadth, weighing about 0.0004 gm. Hatching takes place from early morning to till noon.

Larval stages

Each larval instar of *C. trifenestrata* worm has distinct body colour and tubercular arrangement.

1st instar: The newly hatched larva is yellowish to brown in colour with black tubercles and covered by whitish hair like setae. Head is black with two reddish black spots in the thorax. Anal flap and claspers are creamy yellow in colour. Thoracic legs are black, while abdominal legs are creamy yellow in colour. The larvae which turn pinkish at the later part exhibit colonial habit.

2nd instar: The worms are light pink to maroon in colour with black tubercles which are covered with whitish hair like setae. Head is black while anal flap and claspers are creamy brown in colour.

3rd instar: Black and pinkish striped body is covered by pinkish tubercles with whitish hair like setae. Head, anal flap and claspers are pinkish in colour. Thoracic legs are pinkish-gray while abdominal legs are pinkish with blackish crochets.

4th instar: Colour patterns, tubercles and setae become prominent. Creamy yellow lateral line and spiracles are distinct at this stage. The body is covered with creamy yellow spots and whitish hairs.

5th instar: The body banding colour patterns are more prominent. Head is pinkish gray while anal flap and claspers are pink in colour and are covered with whitish hairs. Creamy yellow colour lateral line and spiracles are very much prominent and distinct at this stage. Abdominal legs are pink in colour with blackish hairs

Pupa and cocoon

The pupa is reddish gray to brownish gray in colour. The pupa generally enters in diapause by undergoing seven months winter hibernation from December to June

TABLE 1. Measurement of developmental stages of *Cricula trifenestrata* Helfer in two host plants

Parameters	Host plants	Stages								
		Egg	1st	2nd	3rd	4th	5th	Pupa	Adult	
								Male	Female	Male	Female
Length (cm) (Mean ± SE)	A	0.18 ± 0.007	0.66 ± 0.09	0.96 ± 0.14	1.40 ± 0.19	4.01 ± 0.43	6.00 ± 0.45	2.62 ± 0.11	2.68 ± 0.13	1.82 ± 0.21	2.33 ± 0.16
	B		0.62 ± 0.07	1.20 ± 0.09	1.54 ± 0.20	3.96 ± 0.54	5.64 ± 0.40	2.22 ± 0.11	2.74 ± 0.10	1.91 ± 0.12	2.36 ± 0.17
	S. Ed ±	0.005	0.087	0.144	0.157	0.107	0.244	0.134	0.189	0.134	0.176
	CD-5%	-	NS	NS	NS	NS	NS	0.372*	NS	NS	NS
Breadth (cm) (Mean ± SE)	A	0.12 ± 0.003	0.12 ± 0.01	0.16 ± 0.02	0.20 ± 0.02	0.59 ± 0.08	0.86 ± 0.09	0.78 ± 0.06	1.12 ± 0.07	0.64±0.05	0.97±0.05
	B		0.12 ± 0.01	0.18 ± 0.03	0.26 ± 0.04	0.61 ± 0.09	0.70 ± 0.10	0.48 ± 0.04	0.78 ± 0.04	0.53±0.05	1.02±0.08
	S. Ed ±	0.002	–	0.031	0.046	0.020	0.068	0.071	0.103	0.087	0.050
	CD-5%	–	–	NS	NS	NS	NS	0.196*	0.286*	NS	NS
Weight (gm) (Mean ± SE)	A	0.003 ± 0.0003	0.029 ± 0.02	0.113 ± 0.01	0.422 ± 0.20	1.690 ± 0.21	2.560 ± 0.27	1.260 ± 0.20	1.778 ± 0.19	0.982±0.04	1.262±0.09
	B		0.033 ± 0.02	0.115 ± 0.01	0.469 ± 0.26	1.990 ± 0.33	2.522 ± 0.23	0.970 ± 0.03	1.636 ± 0.07	0.804±0.10	0.956±0.05
	S. Ed ±	0.0002	0.005	0.027	0.069	0.215	0.248	0.211	0.209	0.115	0.100
	CD-5%	–	NS	NS	NS	NS	NS	NS	NS	NS	0.277*

* = Significant at 5% level of probability, NS = Non significant

A = *Persea bombycina* (Som), B = *Litsea citrata* (Mejankari).

(onset of monsoon) due to prolong winter period in Nagaland. The spindle shaped cocoon is soft, often mesh like and perforated with a thin film or floss layer and long peduncle. The colour is creamy yellow to golden yellow. While Som fed larvae produce golden-yellow cocoons. Mejankari fed cocoons are creamy yellow in colour.

Adult

Ground colour of male moth is light orange, sub-marginal darker, patagia, costal margin, tegulate, thorax and abdomen always similar to ground colour. At the end of the shell a transparent fenestra surrounded with grey brown in colour, a second (rudimentary) fenestra above this, near the costal margin a small grey brown spot. The post median line is distinct and also grey brown in colour. Hind wing colour is also similar to forewing, very small eye spots in the form of a gray brown dot or a tiny fenestra, the antemedian line much distinct and the post median line is wavy. The wing of the female moth has irregular hyaline spots, which are large and variable in number. In male these spots are small and circular. The wing span of the male moth is 56 to 78 mm, forewing is 29 to 36 mm in length and hind wing is 21 to 25 mm in length. Similarly the wing span of the female moth is 75 to 81 mm, forewing is about 39 to 43 mm in length and hind wing is 23 to 28 mm in length.

The species undergoes complete metamorphosis and passes through four stages, viz. egg, larva, pupa and adult. During the moult anterior part of the body remains suspended, the pro-thoracic hood is fully stretched and head is bent ventrally inward. Moths emerge in the evening through the valve of base of the cocoon. Mating occurs on the same day (mid night), usually continue to the following day evening if undisturbed and lay eggs in the same evening after breaking pair. The male moth dies after 4–5 days of copulation and female dies after 6–8 days of egg laying.

Seasonality and effect of host plants on rearing performance

Biology and rearing performance of *C. trifenestrata* in two different host plants exhibited strong seasonality on growth and development in different stages. Summer season (July–September) with the average minimum and maximum temperature of 20.00°C ± 2.08°C and 32.17°C ± 3.21°C respectively was first breeding season for this insect. The relative humidity ranged from 61.66 ± 5.0 to 90.17 ± 2.52%. Second breeding period corresponded to autumn (October–December) season with the minimum and maximum temperature of 10.00°C ± 2.65°C and 26.80°C ± 6.0°C respectively. The relative humidity fluctuated between 49.83 ± 5.03 and 87.84 ± 2.52% (Table 2). The incubation period (days) was shorter during summer on both host plants i.e. *P. bombycina* (13.50 ± 0.17) and *L. citrata* (13.70 ± 0.15) than autumn (14.60 ± 0.16 and 14.80 ± 0.20 respectively) season. Similarly total larval duration was also shorter in *P. bombycina* (40.70 ± 1.56 and 48.50 ± 1.02 days) in comparison to *L. citrata* (46.30 ± 1.72 and 53.20 ± 0.97 days) in summer and autumn season respectively. This was due to decreasing trend of temperature and relative humidity from summer to autumn season. The pupal period was found to be shorter in *P. bombycina* (17.30 ± 0.56 days) than *L. citrata* (19.30 ± 0.50 days) during 1st rearing

TABLE 2. Meteorological data in different rearing seasons

Parameters		Seasons	
		Summer	Autumn
Temperature (°C)	Max	32.17 ± 3.21	26.80 ± 6.0
	Min	20.00 ± 2.08	10.00 ± 2.65
Avg. Humidity (%)	Max	90.17 ± 2.52	87.84 ± 2.52
	Min	61.66 ± 5.0	49.83 ± 5.03
Rainfall/Day (mm)		10.92 ± 4.72	1.21 ± 0.55
Rainy Days (Nos.)/Month		21.67 ± 3.79	4.33 ± 7.57

season, while pupa hibernated for seven months from December to June (i.e. onset of monsoon). Adult longevity was recorded maximum in summer than winter season while feeding on *L. citrata* (Table 3).

The important production parameters, i.e. fecundity (nos.), hatching percentage, effective rate of rearing (ERR) and cocoons per disease free laying was significantly higher when fed on *P. bombycina* than that of *L. citrata*. While fecundity was recorded maximum in *P. bombycina* than *L. citrata* in both seasons, hatching percentage was maximum (71.38 ± 1.32) in *P. bombycina* and minimum (69.98 ± 1.09) in *L. citrata* during first season and second season respectively. Mean effective rate of rearing (38.42 ± 1.41%) and cocoons per disease free laying (22.90 ± 1.24) was higher in *P. bombycina* than *L. citrata*, however, difference was not significant between the seasons (Table 4).

Green cocoon weight of male (1.51 ± 0.14 gm) and female (1.98 ± 0.11 gm) was found to be maximum during first rearing season in *P. bombycina* and it was minimum in male (1.01 ± 0.05 gm) and female (1.78 ± 0.08 gm) in *L. citrata* in 2nd and 1st rearing season respectively, showing the effect of food plants on cocoon characters. Except for significant difference in green cocoon weight and shell ratio of male, no significant difference was observed between the seasons. The interaction effect due to host plant × season was significant only in green cocoon breadth of male and shell weight of female. Maximum shell weight and shell ratio of female was recorded as 0.23 ± 0.02 and 11.95 ± 0.98 respectively on *P. bombycina* while minimum was recorded as 0.16+0.01 and 8.91 ± 0.49 respectively in *L. citrata* during first rearing season. In male, maximum shell ratio (11.00 ± 0.59) was recorded in *L. citrata* during 2nd season while minimum (7.03 ± 0.40) in *P. bombycina* during 1st rearing season. The shell weight of male was almost similar in both food plants between two different seasons (Table 5). Similar observation has also been made by Yadav and Goswami (1992) who studied the nutritional contribution of *M. bombycina* and *L. polyantha* in relation to rearing of muga silkworm (*Antheraea assamensis*). Slanky and

TABLE 3. Seasonality and impact of host plants on life cycle parameters of *Cricula trifenestrata* Helfer

Seasons	Host Plants	Stages (days)								
		Eggs	1st	2nd	3rd	4th	5th	Pupa	Adults	
									Male	Female
Season I (Jul–Sept)	A	13.50 ± 0.17	7.90 ± 0.31	6.80 ± 0.33	6.70 ± 0.21	7070 ± 0.21	11.60 ± 0.50	17.30 ± 0.56	3.80 ± 0.25	6.30 ± 0.30
	B	13.70 ± 0.15	8.70 ± 0.21	7.60 ± 0.22	7.70 ± 0.21	8.90 ± 0.23	13.40 ± 0.85	19.30 ± 0.50	4.20 ± 0.20	8.40 ± 0.40
Season II (Oct–Dec)	A	13.60 ± 0.16	8.60 ± 0.16	7.30 ± 0.16	7.60 ± 0.16	9.00 ± 0.26	15.90 ± 0.28	Diapause	3.60 ± 0.22	5.60 ± 0.22
	B	13.80 ± 0.20	9.30 ± 0.21	8.50 ± 0.17	8.80 ± 0.13	9.60 ± 0.16	17.00 ± 0.30		4.00 ± 0.21	7.60 ± 0.22
Mean ± SE	A	13.05 ± 0.17	8.25 ± 0.19	7.10 ± 0.19	7.15 ± 0.17	8.35 ± 0.22	13.75 ± 0.57	8.65 ± 2.00	3.70 ± 0.16	5.95 ± 0.20
	B	14.25 ± 0.18	9.00 ± 0.16	8.05 ± 0.17	8.25 ± 0.18	9.25 ± 0.16	15.20 ± 0.60	9.65 ± 2.23	4.10 ± 0.14	8.00 ± 0.21
S.Ed ±	Host Plant	0.13	0.20	0.22	0.16	0.20	0.46	0.38	0.19	0.26
	Season	0.13	0.20	0.22	0.16	0.20	0.46	0.38	0.19	0.26
	H × S	0.18	0.28	0.31	0.23	0.29	0.65	0.53	0.27	0.36
CD-5%	Host Plant	NS	0.41***	0.45***	0.32***	0.41***	0.94**	0.78*	0.38*	0.52***
	Season	0.26***	0.41**	0.45**	0.32***	0.41***	0.94**	0.78***	NS	0.52**
	H × S	NS	NS	NS	NS	NS	NS	1.10*	NS	NS

Total Larval Duration (Days)	Host plants	
	A	B
Season-I (June–July)	40.70 ± 1.56	46.30 ± 1.72
Season-II (Oct–Nov)	48.50 ± 1.02	53.20 ± 0.97

* = Significant, ** & *** = Highly significant at 5% level of probability & NS = Non significant.
A = *Persea bombycina* (Som), B = *Litsea citrata* (Mejankari).

TABLE 4. Seasonal variation of production parameters of *Cricula trifenestrata* Helfer in two host plants

Seasons	Host plants	Parameters			
		Fecundity (Nos.)	Hatching (%)	ERR (%)	Cocoons per Disease free laying
Season-I (July–Sept)	A	85.70 ± 4.04	71.38 ± 1.32	43.03 ± 1.04	26.20 ± 1.40
	B	80.80 ± 3.45	71.31 ± 1.08	45.96 ± 0.88	26.50 ± 1.29
Season-II (Oct–Dec)	A	84.30 ± 3.89	70.25 ± 1.15	33.80 ± 1.60	19.60 ± 1.47
	B	77.90 ± 2.91	69.98 ± 1.09	29.67 ± 0.32	16.00 ± 0.86
Mean ± SE	A	85.00 ± 2.73	70.81 ± 0.86	38.42 ± 1.41	22.90 ± 1.24
	B	79.35 ± 2.22	70.65 ± 0.76	37.81 ± 2.02	21.25 ± 1.42
S. Ed ±	Host plant	3.31	1.16	1.25	1.15
	Season	3.31	1.16	1.25	1.15
	Host plant × Season	4.68	1.64	1.76	1.63
CD-5%	Host plant	NS	NS	NS	NS
	Season	NS	NS	2.55***	2.32***
	Host plant × Season	NS	NS	3.61**	NS

** & *** = Highly significant at 5% level of probability, NS = Non significant.
A = *Persea bombycina* (Som), B = *Litsea citrata* (Mejankari).

TABLE 5. Seasonal variation of cocoon parameters of *Cricula trifenestrata* Helfer on two different host plants

Seasons	Host Plant	Parameters: Green Cocoon (Male) L. (cm)	B (cm)	Wt. (gm)	Pd./L. (cm)	Green Cocoon (Female) L. (cm)	B (cm)	Wt. (gm)	Pd./L. (cm)	Shell Wt. (gm) Male	Female	Shell ratio (%) Male	Female
Season I (Jul–Sept)	A	3.82 ± 0.11	1.13 ± 0.05	1.51 ± 0.14	7.62 ± 0.91	4.09 ± 0.07	1.35 ± 0.05	1.98 ± 0.11	6.98 ± 0.89	0.10 ± 0.01	0.23 ± 0.02	7.03 ± 0.40	11.95 ± 0.98
	B	3.39 ± 0.12	0.71 ± 0.05	1.07 ± 0.03	7.90 ± 1.07	3.92 ± 0.11	1.17 ± 0.01	1.78 ± 0.08	6.84 ± 0.63	0.11 ± 0.01	0.16 ± 0.01	9.74 ± 0.78	8.91 ± 0.49
Season II (Oct–Dec)	A	3.69 ± 0.11	1.03 ± 0.04	1.16 ± 0.12	7.06 ± 0.55	4.02 ± 0.10	1.24 ± 0.06	1.89 ± 0.11	7.46 ± 0.60	0.11 ± 0.01	0.20 ± 0.01	9.82 ± 0.77	10.80 ± 0.79
	B	3.61 ± 0.10	0.99 ± 0.07	1.01 ± 0.05	7.78 ± 0.63	4.01 ± 0.09	1.15 ± 0.05	1.84 ± 0.06	6.33 ± 0.52	0.11 ± 0.01	0.18 ± 0.01	11.00 ± 0.59	9.63 ± 0.38
Mean ± SE	A	3.76 ± 0.08	1.08 ± 0.03	1.33 ± 0.10	7.34 ± 0.52	4.06 ± 0.06	1.30 ± 0.04	1.94 ± 0.08	7.22 ± 0.53	0.11 ± 0.01	0.22 ± 0.01	8.42 ± 0.53	11.37 ± 0.63
	B	3.50 ± 0.08	0.85 ± 0.05	1.04 ± 0.03	7.83 ± 0.60	3.97 ± 0.07	1.16 ± 0.03	1.81 ± 0.05	6.59 ± 0.40	0.11 ± 0.01	0.17 ± 0.01	10.37 ± 0.50	9.29 ± 0.31
S.Ed. ±	Host Plant	0.11	0.05	0.09	0.84	0.10	0.05	0.09	0.75	0.01	0.01	0.69	0.75
	Season	0.11	0.05	0.09	0.84	0.10	0.05	0.09	0.75	0.01	0.01	0.69	0.75
	H × S	0.15	0.07	0.13	1.18	0.14	0.07	0.13	1.06	0.01	0.02	0.98	1.06
CD-5%	Host Plant	0.22*	0.11***	0.18**	NS	NS	0.10**	NS	NS	NS	0.03**	1.42**	1.54*
	Season	NS	NS	0.18*	NS	NS	NS	NS	NS	NS	NS	1.42**	NS
	H × S	NS	0.15***	NS	NS	NS	NS	NS	NS	NS	0.04*	NS	NS

* = Significant, ** & *** = Highly significant at 5% level of probability and NS = Non significant.
A = *Persea bombycina* (Som), B = *Litsea citrata* (Mejankari).
L. = length, B = breadth, Wt. = weight, Pd = peduncle, cm = centimeter, gm = gram.

TABLE 6. Seasonal variation of foliar constituents of host plants of *Cricula trifenestrata* Helfer

Seasons	Host plants	Parameters							
		Moisture (%)	Total Carbohydrate %	Total Soluble Sugar (%)	Total Reducing Sugar (%)	Crude Fibre (%)	Total Nitrogen (%)	Crude Protein (%)	Total Ash (%)
Summer	A	69.81 ± 0.34	10.48 ± 0.54	3.32 ± 0.10	1.33 ± 0.06	15.35 ± 0.97	2.67 ± 0.07	16.70 ± 0.45	7.83 ± 0.25
	B	68.59 ± 0.60	14.16 ± 0.39	4.80 ± 0.29	0.31 ± 0.06	20.06 ± 1.60	2.65 ± 0.03	16.56 ± 0.19	3.78 ± 0.06
Autumn	A	64.24 ± 0.64	9.32 ± 0.24	3.73 ± 0.29	1.43 ± 0.12	17.01 ± 1.98	2.60 ± 0.30	16.25 ± 1.84	7.80 ± 0.21
	B	60.81 ± 1.45	12.93 ± 0.31	4.22 ± 0.16	0.39 ± 0.01	16.19 ± 1.66	3.01 ± 0.12	18.82 ± 0.74	4.11 ± 0.09
Mean ± SE	A	67.03 ± 0.49	9.90±0.39	3.35±0.20	1.38±0.09	16.18±1.48	2.61±0.19	16.48±1.15	7.82±0.23
	B	64.70 ± 0.76	13.55±0.35	4.51±0.23	0.35±0.04	18.13 1.63	2.83±0.08	17.69±0.47	3.95±0.08
S.Ed ±	Host plant	0.67	0.51	0.24	0.13	0.15	0.03	0.23	0.22
	Season	1.00	0.06	0.21	0.27	0.29	0.001	0.007	0.12
	H × S	0.30	1.17	0.31	0.05	0.04	0.08	0.55	0.37
CD-5%	Host plant	NS	1.06***	0.51*	NS	NS	NS	0.47*	0.45**
	Season	4.28***	0.26**	0.90*	1.16**	1.25**	NS	NS	0.52*
	H × S	1.30**	NS	1.34**	NS	NS	NS	2.37***	1.59**

* = Significant, ** & *** = Highly significant at 5% level of probability & NS = Non significant.
A = *Persea bombycina* (Som), B = *Litsea citrata* (Mejankari).

Scriber (1985) described that the quality of food consumed by larvae influenced the fecundity, growth rate, development time and survival of adults. While *P. bombycina* had higher moisture content in summer, *L. citrata* possessed higher percentage of total carbohydrate, total soluble sugar and crude fibre than *P. bombycina*. However percentage of total reducing sugar, total nitrogen, crude protein and total ash was higher in *P.bombycina* than *L. citrata*. Except for moisture content, the difference in total reducing sugar, crude fibre and total ash was not significant between the host plants in two seasons (Table 6). Hence, the present investigation indicated that *P. bombycina* and summer season was more suitable for rearing of *C. trifenestrata* than *L. citrata* highlighting the interaction effect of host plants and seasons on production parameters. Evaluation of suitable food plants in relation to growth, development and reproductive potential of other silkworm species like *Bombix mori* Linn. (Saratchandra *et al.*, 1992), *Antheraea assamensis* Ww (Chaudhury *et al.*, 2000), *Antheraea mylitta* Drury (Rath, 2000), *Samia ricini* Donovan (Jayaramaiah and Sannapa, 2000) have also been made earlier. Mulken and Brusven (1962) and Mc Caffery (1975) pointed out that egg production in insects was influenced by the host plant selection. The qualitative and quantitative characters of the cocoon greatly varied and much depended upon the type of food plants used (Sharma *et al.*, 1995). Krishnaswamy *et al.* (1971) also observed that growth, development, and the economic characters of the cocoons were greatly influenced by the nutritional content of mulberry leaves. Rajadurai and Thangavelu (2000) while studying the biology of *Actias selene* in three different host plants observed strong influence of host plants on colour and morphometry of immature stages and adults, life cycle parameters, larval duration, reproductive programming and commercial characters of cocoons.

C. trifenestrata was found to be free from other silk worm diseases like pebrine, muscardine and infestation of uzi fly, ant, and wasp. Preying birds were also negligible. While *P. bombycina* is considered as primary host plants for rearing of *A. assamensis* (muga silk worm) in North Eastern region, *L. citrata* has its importance as secondary host plant. Both the plants are abundantly available through out the state of Nagaland and can be planted for rearing of *C. trifenestrata* along with muga silk worm for commercial exploitation. Due to less exploitation by local sericulture farmers as well as unawareness about the species, the present rich fauna of *C. trifenestrata* may face extinction problem in future. Thus, the critical evaluation of suitable conservation and utilization strategies is urgently required for establishment of this species as resourceful, disease resistant and genetically stable breeding material for flourishing the seri - culture industry along with other silk moths

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Comparison of external genitalia of four species of genus *Spilostethus* Stal (Hemiptera: Lygaeidae)

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ABSTRACT: Species belonging to the genus *Spilostethus* Stal are universally distributed. In this paper external genitalia of four Indian species i.e. *S. pandurus* (Scopoli), *S. hospes* (Fabricius), *S. simla* (Distant) and *S. cous* (Distant) has been compared. A key to these four species incorporating external genitalic features has been provided. Descriptions as well as photographs of each part of both male and female genitalia are provided.

KEYWORDS: *Spilostethus*, external genitalia, key, descriptions.

INTRODUCTION

Spilostethus is a very extensive genus, almost universally distributed, though largely represented in tropical America. According to Fauna of British India Distant (1901, 1902, 1909, 1910, 1911 and 1918) only six species have been reported from British India. For this genus a number of subgenera have been proposed. The species found in British India, however, are of a typical and little divergent character. This genus is diagnosed by pronotum either without a median longitudinal keel or with a keel not reaching anterior margin; posterior margin straight before scutellum, scutellum depressed, with a longitudinal median keel commonly joined to a median or premedian transverse ridge; metapleura with posterior margin straightly or somewhat roundly truncate not oblique, the anterior and posterior margins of this nearly parallel; corium almost or quite impunctate, its posterior margin straight; membrane not at all or usually narrowly but never with the apex more widely white margined, rarely entirely or for the most part clear of whitish; head with a red or pale spot or longitudinal fascia near base; eyes in contact with the anterior margin of pronotum. Species mostly black marked with red or sometimes with the later color predominating. Species feed mainly on wild vegetation.

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MATERIALS AND METHODS

Bugs were collected by picking them individually and by beating vegetation and branches of trees below which a white sheet was spread. After collection the bugs were killed with ethyl acetate vapours and pinned, stretched and kept in wooden boxes for further examination. For dissection of male and female genitalia methodology given by Ashlock (1957) was used, the dried specimens were relaxed in a wet pertidish for few hours. Their abdomens were separated from the whole insect and were kept in 10% KOH at 70° temperature for 40–80 minutes depending upon the size of the insect. After dissections the structures were washed in distilled water and then dehydrated through ascending grades of alcohol, cleared in clove oil and preserved. Photography was done using image processing unit located in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala. Orientation of the genital structures was arranged in such a way that it clearly depicted its distinctive features. All the measurements have been made under stereozoom binocular microscope fitted with a graph eye piece (ocular grid).

Genus *Spilostethus* Stal

Lygaeus, Fabricius (pro parte) 1794, *Ent. Syst.*, 4: p. 133.

Lygaeosoma, Fieb. (pro parte) 1861, *Eur. Hem.* pp. 45, 167.

Lygaeodon, Puton 1869, *Ann. Soc. Ent. Fr.*, p. 139.

Subg. *Melanospilus, Stalagmostethus, Spilostethus, Graptolomus, Micropsilus, Melanostethus, Melanerythrus*, Stal 1868, *Hem. Fabr.*, pp. 1, 72, 73, 75, 76.

Subg. *Cosmopleurus, Melanocoryphus*, Stal, 1872, *Ofv. Vet.-Ak. Forh.*, 7: p. 41.

Subg. *Haemobaphus, Melanopleurus, Craspeduchus, Ochrostomus, Ochrimnus*, Stal, 1874, *En. Hem.*, 4: pp. 104, 105, 113.

Subg. *Eulygaeus*, Reuter, 1888, *Act. Soc. Fenn.*, 15: p. 180.

Subg. *Tropidothorax*, *Bergroth*, 1894, *Ann. Soc. Ent. Belg.*, 38: p. 547.

Lygaeus: Distant 1902, *Fauna Brit. Ind.*, 2: p. 5.

Spilostethus, Oshanin 1912, *Kat. Palaarkt., Hemipt.*, p. 27.

TYPE SPECIES: *Spilostethus pandurus* (Scopoli).

Key to the studied species of genus *Spilostethus* Stal

1 Scent gland black; spermatheca with ovoid bulb, spermathecal duct broad; pygophore broader and less sclerotized, hair denser; paramere with inner process 1 2

– Scent gland red; spermatheca with long tube like bulb, spermathecal duct narrow; pygophore narrow and much sclerotized, hair less; paramere with inner process smaller 3

2 Fascia on pronotum grayish yellow with black margins; corium red with two black spots; hemelytral membrane with white spots. Bulb broad and short with round margins, spermathecal duct thin and long; paramere stem with anterior process broad, posterior process very short and round, blade thicker with round apex; pygophore broad and flattened, posterior margin sinuate ..... *pandurus* (Scopoli)

– Fascia on pronotum black; corium with suffusion of red and black; hemelytral membrane without white spots. Bulb less broad with sinuate margins, spermathecal duct thicker and shorter; paramere stem with anterior process short and triangle shaped, posterior process very broad, blade flattened and thinner with round apex; pygophore spherical, posterior margin round with a small inflexion ..... *hospes* (Fabricius)

3 Head red with black posterior region; rostrum reaching near posterior coxae; prosternum, mesosternum and metasternum red, each with a black fascia; body oval; antennae longer. Bulb long, tubular and coiled, spermathecal duct divided in two parts, anteriorly thin and posteriorly thick; blade anteriorly broad and narrow posteriorly with substraight apex, anterior process less prominent; pygophore broad, squarish with posterior margin sinuate ..... *simla* (Distant)

– Head black with lateral areas red; rostrum reaching near mesocoxae, prosternum black except anterior region, lateral area near legs red, mesosternum and metasternum black except area near legs; body elongate; antennae shorter. Bulb broad and shorter, tubular and coiled, spermathecal duct not divided; blade with sinuate margin with apex broad and subround , anterior process more prominent; pygophore with round posterior margin ..... eous (Distant)

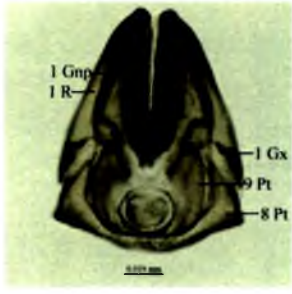
DESCRIPTIONS OF THE GENITALIA

Spilostethus pandurus (Scopoli)

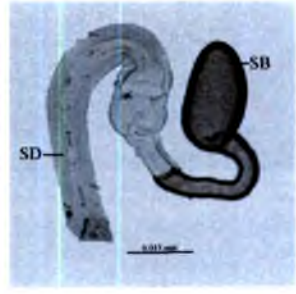
Male genitalia: Pygophore (Fig. 3) with anterior margin sinuate with a concavity, lateral outer margin subround, posteriorly broad, posterior margin sinuate with median projection projecting outside, long hair on posterior side, in lateral view anterior margin subround; paramere (Fig. 4) with stem long, outer and inner margins sinuate, inner projection broad, subround, but not projecting outside, outer projection squarish and with short hair, blade thick, long and highly curved with subround apical margin; aedeagus (Fig. 5) with phallosoma long and wide with more sclerotization in lateral region, conjunctiva without any appendage, vesica thick in form of irregular spirals, ejaculatory reservoir complete without fusion of parts, gonoporal process coiled and of equal breadth, ring sclerite at middle.

Female genitalia: (Fig. 1) 1st gonocoxa triangular, 1st gonapophysis broad with subround apex, 8th paratergite more or less triangular with outer and inner margins sinuate, 9th paratergite long, rectangular; spermatheca (Fig. 2) with long spherical bulb, duct divided in to two parts, anterior part long and thin, posterior part short.

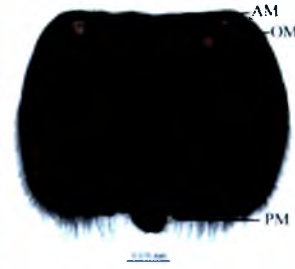
Spilosthus pandurus (Scopoli)



1. Genital Plate

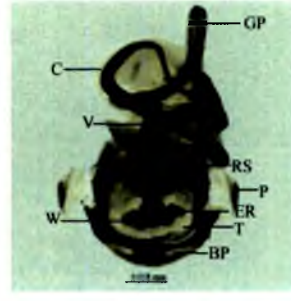


2. Spermatheca



3. Pygophore

4. Paramere



5. Aedeagus

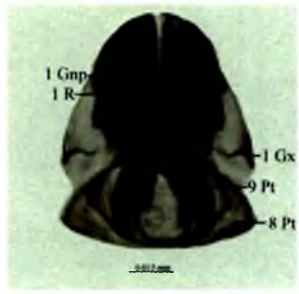
FIGURES 1–5. *Spilostethus pandurus*. 1. Genital plate, 2. Spermatheca, 3. Pygophore, 4. Paramere, 5. Aedeagus.

Spilostethus hospes (Fabricius)

Male genitalia: Pygophore (Fig. 8) round with anterior margin subround, lateral outer margin round, posterior margin sinuate with a median inexion in the form of two lobes, in lateral view anterior margin subround, dorsal margin sinuate; paramere (Fig. 9) with stem broad, inner margin and outer margin sinuate, inner projection squarish, outer margin broad and squarish with short hair, blade broad and curved with subround apex, outer and inner margins substraight, long hair on blade; aedeagus (Fig. 10) with phallosoma long and wide, strongly sclerotized on lateral sides, conjunctiva without any appendage, ejaculatory reservoir complete without fusion of parts, wings broad, vesica thick and irregularly coiled, gonoporal process less coiled, ring sclerite present at middle, basal plate with pivot.

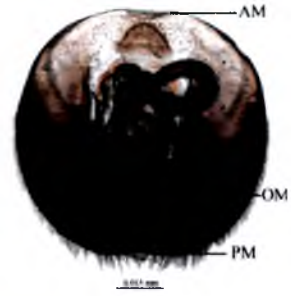
Female genitalia: (Fig. 6) 1st gonocoxa large and triangular, 1st gonapophysis broad with round apex, 8th paratergite triangular with outer margin subround and inner margin sinuate, 9th paratergite triangular, broader than 8th; spermatheca (Fig. 7) with bulb long, tubular and broad, apically narrow, tube divided in two parts, distal part curved, short and thin, proximal part thick, longer than distal.

Spilosthus hospes (Fabricius)



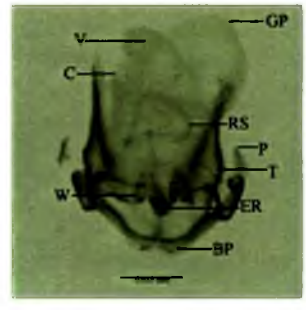
6. Genital Plate

7. Spermatheca



8. Pygophore

9. Paramere



10. Aedeagus

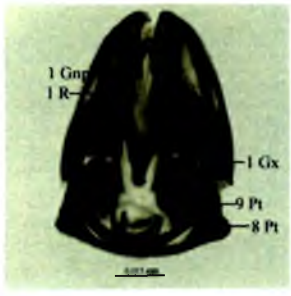
FIGURES 6–10. *Spilostethus hospes*. 6. Genital plate, 7. Spermatheca, 8. Pygophore, 9. Paramere, 10. Aedeagus.

Spilostethus simla (Distant)

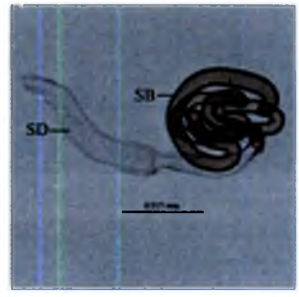
Male genitalia: Pygophore (Fig. 13) almost squarish with anterior margin sinuate, outer anterolateral margin substraight, posteriorly sinuate, posterior margin with inexion, divided in three lobes, two lateral lobes and with a sinuate lobe, in lateral view anterior margin substraight; paramere (Fig. 14) with stem long and substraight, outer margin straight, inner margin concave, inner projection short and triangular, outer projection long and squarish with long hair, blade narrow toward apex with substraight margin, outer and inner margins substraight, long hair on blade; aedeagus (Fig. 15) with phallosoma broad and long, laterally much sclerotized, conjunctiva long with a pair of lateral lobes, ring sclerite present, much broad and complete, vesica short and tightly coiled, covered with membrane, ejaculatory reservoir complete.

Female genitalia: (Fig. 11) 1st gonocoxa triangular, 1st gonapophysis broad with subround apex, 8th paratergite triangular with outer margin substraight and inner margin concave, 9th paratergite squarish; (Fig. 12) speramtheca with long tubular bulb of equal breadth, highly pigmented and continuing with duct, duct divided in to two parts-distal part transparent and thin, proximal part broad, longer than distal.

Spilosthus simila (Distant)



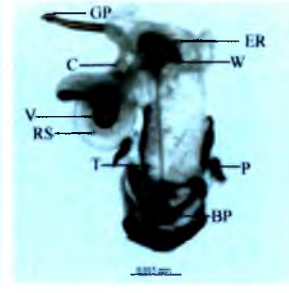
11. Genital Plate



12. Spermatheca

13. Pygophore

14. Paramere



15. Aedeagus

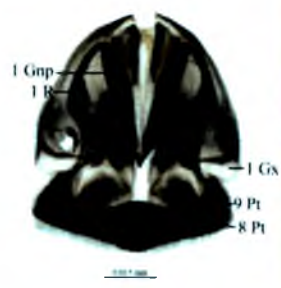
FIGURES 11–15. *Spilostethus simla*. 11. Genital plate, 12. Spermatheca, 13. Pygophore, 14. Paramere, 15. Aedeagus.

Spilostethus eous (Distant)

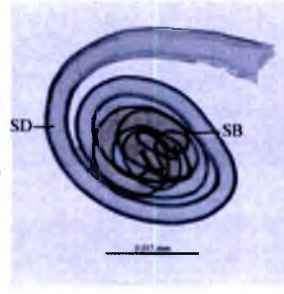
Male genitalia: Pygophore (Fig. 18) almost round with anterior margin sinuate, outer margin round, posterior margin round, large hair on posterior region, in lateral view anterior margin attened and substraight; paramere (Fig. 19) with stem long and squarish, outer and inner margins straight, inner projection small, round, outer projection long with small hair, blade straight, anteriorly broad, narrow at apex, apex subround, inner margin highly sinuate, outer margin less sinuate; aedeagus (Fig. 20) with phallosoma short and broad, much sclerotized toward lateral sides with a pair of lateral lobes, conjunctiva with a pair of sclerotized lobes, ring sclerite present, ejaculatory reservoir complete, wings broad, vesica with two coils, gonoporal process loosely coiled, second gonopore with apical process.

Female genitalia: (Fig. 16) 1st gonocaxa squarish, 1st gonapophysis much broad with acute edge, 8th paratergite triangular with outer margin sinuate, inner margin substraight and 9th paratergite subround; spermatheca (Fig. 17) with bulb long and tubular with round apex, duct long and highly coiled.

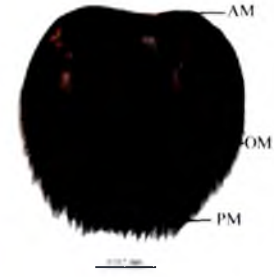
Spilosthus eous (Distant)



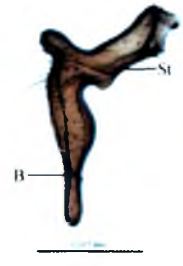
16. Genital Plate



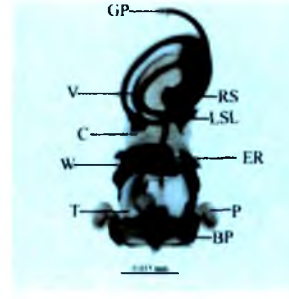
17. Spermatheca



18. Pygophore



19. Paramere



20. Aedeagus

FIGURES 16–20. *Spilostethus eous* 16. Genital plate, 17. Spermatheca, 18. Pygophore, 19. Paramere, 20. Aedeagus.

List of abbreviations used

AM – Anterior Margin, B – Blade, BP – Basal Plate, C – Conjunctiva, ER – Ejaculatory Reservoir, Fig. – Figure, Gnp – Gonapophysis, GP – Gonoporal Process, Gx – Gonocoxa, LSL – Lateral sclerotized lobe, mm – Millimeters, MP – Median Projection, OM – Outer Margin, P – Pivot, PM – Posterior Margin, Pt – Paratergite, R – Ramus, RS – Ring Sclerite, SB – Spermathecal Bulb, SD – Spermathecal Duct, St – Stem, T – Theca, V – Vesica, W – Wing.

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Distant W. L. (1909) Oriental Rhynchota Heteroptera. Ann. Mag. Nat. Hist., 8(3): 491–507.

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Effect of diflubenzuron, a chitin synthesis inhibitor on mortality of *Pericallia ricini* Fabr.

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ABSTRACT: Laboratory experiments have been carried out to evaluate effect of diflubenzuron, an insect growth regulator on 3rd and 5th instar larvae of *Pericalli ricini*. The effect on mortality during moulting, metamorphosis and direct toxic effect has been evaluated by treating the larvae through oral and contact (residual film). Five concentrations viz. 0.1, 1.0, 10, 50 and 100 ppm were tried with oral treatment on both 3rd and 5th instar larvae. Similarly five concentration viz. 0.002, 0.02, 0.2, 1.0 and 2.0 $\mu g/cm^2$ was tried with residual film treatment to same stages of larvae. The corrected per cent of total mortality by oral treatment of third instar varied 22–98 per cent with 0.1 to 100 ppm concentration of insecticide and LC_{50} value was recorded 13.7 ppm. Similarly for 5th instar, the corresponding figure of mortality varied from 25–91.66 per cent with same dose but LC_{50} value based on corrected per cent of total mortality was recorded 10.96 ppm. In respect of residual film treatment to third instar larvae, the corrected per cent of mortality varied 8.33 to 85.83 per cent with 0.002 to 2.0 $\mu g/cm^2$ and LC_{50} value was 0.6 $\mu g/cm^2$. Similarly for fifth instar the corrected mortality varied from 10.2 to 89.79 per cent and LC_{50} was recorded 0.52 $\mu g/cm^2$. The result thus indicated that diflubenzuron is an effective insect growth regulator on *P. ricini* affecting moulting and metamorphosis causing substantial mortality of test insect.

KEYWORDS: *Pericallia ricini*, diflubenzuron, chitin biosynthesis inhibitor, mortality

INTRODUCTION

Pericallia ricini, commonly known as Black hairy caterpillar is a serious pests of oil seed plants, castor and cucurbitaceous crops. The female moths lay eggs in large number on the lower surface of leaf. The larvae feed on young and full grown plant leaves and fruits. In heavy infestation only stems and branches are left behind.

In past many research workers attempted to study the effect of chitin biosynthesis inhibitor on mortality of insect pests and reported almost complete lethal effect of this chemical (Gupta and Verma, 1992; Beninger and Arnason, 1993; Baringbing and

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Karmawati, 1992) but the adequate literature is still lacking on the loss of crop by these pests which survive after exposure with these chemicals. Hence the objective of the present research was to assess the effective dose which causes substantial mortality of this species.

MATERIALS AND METHODS

The eggs of *Pericallia ricini* were collected from castor leaves and reared in the laboratory on castor leaves. Eggs were kept between two leaves in the wooden cages. (60 × 60 × 45 cm) and fresh, clean fleshy leaves were provided daily. After hatching, larvae started feeding on fresh leaves, the excreta and other waste were removed daily from cages. For the protection of larvae from ants, the rearing cages were placed on water filled pots (Earthen cups). Larvae were reared till it metamorphosed into pupa. Freshly emerged adults were transferred to separate jars for oviposition. Honey mixed sugar solution (10%) soaked in a cotton ball, was provided in the plastic cavity (2 × 2 × 1 cm) for feeding the adults. Fresh castor leaves were placed in the glass chimney for egg laying. The females laid eggs on leaf surface. Such leaves along with eggs were transferred into another glass jar. The eggs were kept between fresh succulent castor leaves to provide food for hatching larvae easily and also to prevent leaves from rapid evaporation. Larvae were takenout from the cages and were starved for atleast six hours in order to avoid much variation in the results. The larvae fed on caster leaves, were dipped in different concentrations of insecticide emulsion. Corrected mortality was calculated by using Abbott's formula (1925).

$$\text{Per cent corrected mortality} = \frac{(\%\ \text{mortality in test} - \%\ \text{Mortality in control})}{100 - \%\ \text{Mortality in normal}} \times 100$$

RESULTS AND DISCUSSIONS

Oral treatment

Treatment to 3rd instar larvae (Table 1) showed that the number of larvae moulted to 4th and 5th instar decreases with increasing concentration but the effect is not manifested during moulting from 3rd to 4th instar with the minimum concentration tried (0.1%). The mortality of insect from 3rd to 4th instar and 4th to 5th instar varied 0–7.4 per cent and 0.4–1.2 per cent respectively. The per cent mortality was more during moulting to 4th instar than that of to 5th instar with all the concentration tried in the experiment. The number of larvae pre-pupated and pupated also decreases with increasing concentration. The total mortality during larval and pupal stage was recorded with 50 and 100 ppm as none of the test insect reached to adult stage. The total mortality of insect thus varied 22–98 per cent and based on corrected mortality, the LC_{50} was recorded as 13.7 ppm.

In respect of treatment of 4th instar larvae (Table 2), the number of larvae pre-pupated decreases with increasing concentration and similar observation was recorded

TABLE 1. Response of oral treatment of diflubenzuron on third instar larvae of *P. ricini*

Concentration (ppm)	Average no. of larvae treated	Average no. of larvae moulted to 4th instar	Average no. of larvae moulted to 5th instar	Average no. of larvae larvae pre-pupated	Average no. of larvae pupated	Average no. of adults emerged	Total mortality from larvae to adult	Corrected mortality (%)
0.1	10	10 (0.0)	9.6 (0.4)	9.0 (0.6)	9.0 (0.0)	7.8 (1.2)	2.2 (22.0)	22.0
1.0	10	7.8 (2.2)	7.6 (0.2)	7.6 (0.0)	7.0 (0.6)	7.0 (0.0)	3.0 (30.0)	30.0
10	10	6.4 (3.6)	5.2 (1.2)	4.4 (0.8)	3.2 (1.2)	2.4 (0.8)	7.6 (76.0)	76.0
50	10	4.2 (5.8)	3.6 (0.6)	2.8 (0.8)	1.6 (1.2)	0	8.4 (84.0)	84.0
100	10	2.6 (7.4)	2.4 (0.2)	0.8 (1.6)	0.2 (0.6)	0	9.8 (98.0)	98.0
Control	10	10 (0.0)	10 (0.0)	10 (0.0)	10 (0.0)	0	0.0 (0.0)	-

Note: Figures in parentheses indicate mortality during moulting or metamorphosis.

TABLE 2. Response of oral treatment of diflubenzuron on fifth instar larvae of *P. ricini*

Concentration (ppm)	Average no. of larvae treated	Average no. of larvae pre-pupated	Average no. of larvae pupated	Average no. of adults emerged	Total mortality from larvae to adult	Corrected mortality (%)
0.1	10	8.2 (1.8)	7.8 (0.4)	7.2 (0.6)	2.8 (28.0)	25.00
1.0	10	7.6 (2.4)	6.2 (1.4)	5.4 (0.8)	4.6 (46.0)	43.75
10	10	4.8 (5.2)	4.2 (0.6)	3.6 (0.6)	6.4 (64.0)	62.50
50	10	2.2 (7.8)	1.8 (0.4)	1.2 (0.6)	8.8 (88.0)	87.50
100	10	1.8 (8.2)	1.6 (0.2)	0.8 (0.8)	9.2 (92.0)	91.66
Control	10	9.6 (0.4)	9.6 (0.0)	9.6 (0)	0.4 (4.0)	-

Note: Figures in parentheses indicate mortality during moulting or metamorphosis.

TABLE 3. Response of contact treatment of diflubenzuron on third instar larvae of *P. ricini*

Concentration ($\mu g/cm^2$)	Average no. of larvae treated	Average no. of larvae moulted to 4th instar	Average no. of larvae moulted to 5th instar	Average no. of larvae larvae pre-pupated	Average no. of larvae pupated	Average no. of adults emerged	Total mortality from larvae to adult	Corrected mortality (%)
0.002	10	9.8 (0.2)	9.0 (0.8)	8.8 (0.2)	8.8 (0.0)	8.4 (0.4)	1.2 (12)	8.33
0.02	10	9.2 (0.8)	8.2 (1.0)	8.2 (0.0)	8.0 (0.2)	7.8 (0.2)	2.2 (22)	18.75
0.2	10	7.4 (2.6)	5.6 (1.8)	5.0 (0.6)	4.8 (0.2)	4.0 (0.8)	6.0 (60)	58.33
1.0	10	4.4 (5.6)	4.2 (0.2)	3.6 (0.6)	3.2 (0.4)	1.8 (1.4)	8.2 (82)	81.25
2.0	10	2.6 (7.4)	2.4 (0.2)	2.2 (0.2)	1.0 (1.2)	nil	9.0 (90)	85.83
Control	10	10	10	9.6 (0.4)	9.6 (0.0)	9.6 (0.0)	0.4 (4.0)	-

Note: Figures in parentheses indicate mortality during moulting or metamorphosis.

TABLE 4. Response of contact treatment of diflubenzuron on fifth instar larvae of *P. ricini*

Concentration ($\mu g/cm^2$)	Average no.of larvae treated	Average no. of larvae pre-pupated	Average no. of larvae pupated	Average no. of adults emerged	Total mortality from larvae to adult	Corrected mortality (%)
0.002	10	9.8 (0.2)	9.0 (0.8)	8.8 (0.2)	1.2 (12)	10.2
0.02	10	7.4 (2.6)	6.6 (0.8)	6.4 (0.2)	3.6 (36)	34.69
0.2	10	5.4 (4.6)	4.6 (0.8)	4.2 (0.4)	5.8 (58)	57.14
1.0	10	5.6 (4.4)	4.8 (0.8)	2.2 (2.6)	7.8 (78)	77.55
2.0	10	1.8 (8.2)	1.0 (0.8)	0 (0.0)	9.0 (90)	89.79
Control	10	9.8 (0.2)	9.8 (0.0)	9.8 (0.0)	0.2	-

Note: Figures in parentheses indicate mortality during moulting or metamorphosis.

during pupation stage. However, contrary to 3rd instar larval treatment, the adult emergence also took place with 50 and 100 ppm concentration. As in case of 3rd instar larval treatment, relatively higher mortality was found at initial stage of treatment. The corrected per cent of mortality varied 25–91.67 per cent and the LC_{50} value has been recorded as 10.96 ppm. The number of adults emerged were nil with 50 and 100 ppm in case of 3rd instar larval treatment while corresponding figure for 4th instar treatment was 1.2 and 0.8.

Contact treatment

With regard to treatment of 3rd instar (Table 3), the mortality was found higher during moulting to 4th instar rather than 5th instar with higher concentration (0.2, 1.0 and 2.0 $\mu g/cm^2$). However, with lower concentration (0.002 and 0.02), higher mortality was observed during moulting to 5th instar. The corrected mortality varied 8.33 to 85.83 per cent with 0.002–2.0 $\mu g/cm^2$. The LC_{50} value with corrected per cent of mortality was recorded 0.63 $\mu g/cm^2$.

In respect of treatment of 5th instar (Table 4), with higher concentration of diflubezuron (0.02 to 2.0 $\mu g/cm^2$), the mortalty of treated instar was relatively more (2.6 to 8.2 per cent) than mortality of pupal stage (0.8 per cent) at the corresponding concentration. Highest concentration (2.0 $\mu g/cm^2$) completely stop adult emergence. The corrected mortality of insect varied 10.2 to 89.79 per cent and LC_{50} value 0.52 $\mu g/cm^2$. The comparision of LC_{50} values in respect of oral and contact treatment for both 3rd and 5th instar larvae indicated relatively higher LC_{50} values for 3rd instar larvae treatment.

The relatively high mortality percentage in earlier instar may be due to the disruption of chitin biosynthesis and the result is manifested during moulting of 3rd to 4th instar and at subsequent stages.. Similar result has been reported by Ahmad (1992) on *D. cingulatus* due to application of dimilin and noted this compound caused mortality in nymphs and a significant reduction in fecundity, fertility and progeny development of females emerging from treated nymphs. The reduction was more pronounced in fifth instar treated nymphs than in fourth instar nymphs. In present findings, diflubenzuron used was proved to be highly toxic in these insects (Su and Scheffrahn, 1994; Savitz *et al.*, 1994; Willrich and Boethel, 2001; Smagghe *et al.*, 2004; Chebira *et al.*, 2006; Soltani and Delachambre, 2009; Merzendorfer *et al.*, 2012; Bitshadze, 2013; Katherine *et al.*, 2013 *et al.* etc) as it increases the death rate. Hence this chemical may be successfully used in the field of pest control to keep their population of pests below economic injury level.

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Larvicidal properties of hexane extract of seeds of *Madhuca indica* Linn. (Family: Sapotaceae) against vector species of mosquitoes

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ABSTRACT: Hexane extract of seed of *Madhuca indica* was evaluated for its mosquito larvicidal properties against the larvae of three vector species of mosquito under laboratory conditions. This extract showed strong larvicidal properties, cent percent mortality in the third instar larvae was observed in the bioassay test with *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* at a concentration of 1.0, 1.0 and 2.0% respectively. The LC_{50}, LC_{90} values estimated for 3rd instar larvae *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* after 24 hours of exposure period were 0.08, 0.1 and 0.1% and LC_{90} values were 0.8, 0.81 and 0.8% respectively. It is observed that the essential oil of seed is more effective against *An. Stephensi* (0.08). *Madhuca indica* therefore, can be considered as a probable source of phytochemical used for the development of larvicidal agents against disease vectors.

KEYWORDS: plant products, *Madhuca indica*, larvicidal, mosquito vectors

INTRODUCTION

Plants are rich sources of natural products that can be utilized in the development of environmentally safe methods for insect control. Wide spread use of synthetic insecticides both in public health and agricultural sectors resulted in the development of insecticide resistance in vector species of mosquitoes against insecticides which are commonly used in the public health programmes. Moreover, the indiscriminant uses of these insecticides pose threat to the environment by increasing the problems of air and water pollution. The interest in this field has increased tremendously to search

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for less toxic, eco-friendly, bio-degradable phytochemicals for vector control. Due to environmental concern on the use of synthetic chemicals for vector control and risk of wide spread development of insecticide resistance in disease vectors, the use of environment friendly natural products are required to be encouraged in vector control programmes (Mittal and Subbarao, 2003).

The present communication deals with the laboratory evaluation of mosquito larvicidal properties of hexane extract of seed of *Madhuca indica* plant. *Mathuca indica* Linn. (Family: Sapotaceae) is locally named as *Mahua* (Hindi), *Mahwa* (Bengali), *Ippa* (Telgu), *Mahuda* (Guj) grow in tropical environments around the world, and occurs in all the plains and lower hills of India and common in sub mountain regions of the Himalayas (CSIR Report, 1986). Its seed cake and seed oil is used as manure; it possesses anti-earth worm and laxative properties (CSIR Report, 2007; Seshagiri and Gaikwad, 2007). It is also reported to have toxic chemical allatoxine in the oil of *Madhuca indica* seed (Sidhu *et al.*, 2009). Its bark has been used against ulcer, bleeding and diabetes (Khare, 2000; Useful plants of India, 1992; Dahake Ashok and Chiratan, 2010). Study has reported that the ethanolic extract of this plant recorded 100% mortality in Mustrard Aphids (Rani Manju and Sharma Sunita, 2005). However, no reports on the larvicidal activity of this plant against mosquito vectors. The present study aimed to evaluate the possible anti-larval potential of the hexane extract of *Madhuca indica* against the major vector species of mosquitoes.

MATERIALS AND METHODS

Hexane extract of seed: Fresh seed were collected and dried in shade and powdered. The powdered seed material of this plant (500 kg) was subjected to hexane extraction in a soxhlet apparatus in the ratio of 1 : 5 (w/v) of 95% at room temperature. The extract was made in solvent free and the final residue of hexane extract of *Madhuca indica* obtained was kept at −20 °C till its use for the testing anti-larvicidal activity. For the preparation of test concentrations the extract was volumetrically diluted in alcohol along with emulsifying agent Tween 80 at an appropriate test concentration.

The 3rd instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* were used in this study. Laboratory reared 3rd instar larvae of *An.* stephensi, *Cx. quinquefasciatus* and *Ae. aegypti* were exposed to different concentrations of hexane extract. The test was conducted under laboratory conditions at the room temperature of 27 ± 2°C. Different concentrations of extract was prepared fresh and used for the test. Six concentrations of hexane extract of seed at the range of 0.06–2% were prepared for the testing. The known quantity of hexane extract of seed was re-dissolved in alcohol to make 10% (w/v) stock solution and the required concentration was prepared in double distilled water. The control tests were supplemented with the equal amount of alcohol used for the experiment. For each concentration, at least four replicates, comprising of 25 larvae in each test were exposed. The results scored after 24 hours of continuous exposure period to the test solution and expressed as percent mortality.

TABLE 1. The dosage mortality response of hexane extract of *Madhuca indica* against the larvae of vector species of mosquitoes

Mosquito species	Hexane extract		
	LC_{50} values (%)	LC_{90} values (%)	$X^{2(df=4)}$
Anopheles stephensi	0.08	0.8	7.81
Culex quinquefasciatus	0.1	0.81	12.69
Aedes aegypti	0.1	0.8	6.03

X^2-Chi square, df-degrees of freedom.

The data obtained were subjected to probit analysis to calculate the lethal concentration required to kill 50% and 90% (LC_{50} and LC_{90} values) of the population exposed (Finney, 1976). The test was discarded and repeated when control mortality recorded more than 20%, and when the control larval mortality recorded between to 5–20%, the Abbott's formula was used to calculate the corrected mortality (Abbott, 1925).

The results of the dosage mortality responses of hexane extract of seed of *Madhuca indica* are given Table 1. The LC_{50} values of hexane extract of seed against 3rd instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were 0.08, 0.1 and 0.1% respectively. LC_{90} values recorded for 3rd instar larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were 0.8, 0.81, and 0.8% respectively (Table 1). Cent percent larval mortality against hexane extract of seed was obtained at the concentration of 1.0, 1.0 and 2.0% in the larvae of *An. stephensi*, *Cx. quinquefasciastus* and *Ae. aegypti* respectively. The study revealed that the hexane extract is more toxic to the larvae of *An. stephensi* and *Cx. quinquefasciatus* as compared to *Ae. aegypti*. Variations in the toxicity of phytochemical compounds on target species have been reported due to variations in plant parts used the solvent extraction, geographical origin of the plant, photosensitivity of some of the compounds in the extract etc. (Sukumar *et al.*, 1991). Studies also reported about the variations in the responses of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* species of mosquitoes against the crude extract of *Yucca aloifolia* Linn (Thomas *et al.*, 1994).

Studies on the natural plant products for their efficacy as larvicide during the last decade indicated that the possibility for the development of alternatives to synthetic chemical insecticide. However, more concerted efforts are required to make these environmental friendly compounds viable for their field use in large scale (Kalyanasundaram and Babu, 1982). *Madhuca indica* therefore, can be considered as a probable source of biologically active compounds used in the development of mosquito larvicidal products as they are widely cultivated in rural areas.

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Allatostatin–quantum dot ligands as efficient immunolabelling bioconjugants

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ABSTRACT: Insect allatostatin neuropeptides modulate the secretary pattern of Juvenile hormone in arthropod organisms. Arthropod hormones, neuropeptides and related enzymes all regulate morphogenesis and several physiological functions. Quantum dots (QD) are semiconductor nanocrystals that have unique fluorescent properties. Engineered nanoparticles which have unique properties arising from their small size and their large surface to volume ratio, propose them a robust candidate as sensors in medicine and biotechnology. Fluorescent semiconductor nanocrystals known as quantum dots (QD) facilitate the long term tracking of labeled live cells and molecules. Allatostatins are a ubiquitous family of peptide hormones present in invertebrates and function to inhibit the biosynthesis of JH, activate invertase and ∝-amylase reactions, and block muscle contractions. They also have sequence similarity with somatostatin/galanin, growth regulatory receptors. Anti-cancer drugs based on somatostatin analogues that target G-protein coupled somatostatin receptors, antagonize mitotic functions of growth factors and stimulate tyrosine phosphatases. Stimulation of SSTRs induces apoptosis and control cell proliferation. Biotinylated allatostatin conjugated with CaSe–ZnS QDs was easily permeated inside the cephalic secretary neuronal nucleus of *Anopheles* mosquitoe Ring Glands by endocytosis. Confocal imaging of the treated mosquito brain tissue hence offers an easy intracellular delivery mechanism into living cells were visualized using a Confocal Laser scanning microscope and so the allatostain neuropeptide could be projected as an efficient carrier peptide to transport guest molecules of therapeutic importance to the cytoplasm and nucleus of living cells without any adverse effect on the physiology of the cell.

KEYWORDS: Quantum dots, Allatostain, *Anopheles* mosquito, Confocal Laser Scanning Microscope, Ring Glands.

Live cell imaging is the approach of non-invasively analyzing dynamic processes in the metabolically active living cells utilizing confocal laser scanning microscopic live cell imaging. Many of the fundamental questions in biology can be addressed by providing

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such a non-invasive and highly sensitive approach for probing real time biomolecular interactions in living cells. Such a direct real-time monitoring of single molecule dynamics *in vivo* offers biologists an attractive tool to elucidate the spatio-temporal distribution of bio-molecules in their native environment when all cellular circuits are wired up. In this context less harmful nanoparticles as CdSe (QD) and several of the novel nanobiomolecules as allatostatin neuropeptide can effectively addressed in solving many of the treatment strategies. Hence engineered nanoparticles offer unique properties like their small size, large surface to volume ratio (Jin *et al.* 2011). Their photophysical properties are highly dependent on the chemical composition, shape, and size all of which can be controlled during synthesis. Quantum dots (QD) are nanoparticles with narrow, very specific, stable emission spectra.

The unique wide absorption spectra and narrow emission band that can be tuned from UV to infrared wavelength distinguish them from conventional organic fluorophores and provide many advantages over multi-color fluorescent imaging. The orders of magnitude of QD are much more photo stable than the common organic dyes and fluorophores making them very attractive as a candidate for long term single particle tracking experiments in live cells obtaining long tracking trajectories. The absorption band of typical organic fluorophores is narrow and the emission peak is broadened on the red. On the contrary QD have a broad band absorption spectrum and symmetric narrow emission peak. The ultra high affinity streptavidin–biotin is the most commonly used binding pair, utilizing commercially available streptavidin QD. Such unique properties of QD provide a category cellular imaging probes because of their relatively larger size. QD are best utilized when tagging sparsely distributed target molecules and when long time observation is desired. Bio-conjugation of QD for biological fluorescent labeling is due to their unique physical and optical properties as compared to organic fluorescent dyes. QD is advantageous especially in studying complex membrane organization since it allows the acquisition of diffusion trajectories much longer and much more informative than those obtained with conventional and rapidly photo-bleached fluorophores. Intracellular delivery of QD depends on many factors such as the size, surface charge and surface functionality. QD also lifts the limit of general photodynamic therapy to nuclear-specific photodynamic therapy. Hence transportation of photo sensitizers inside the nucleus and nucleus-specific photo-activation would be highly efficient in the next generation photodynamic therapy strategy since singlet oxygen radicals formed at the cell membrane and inside the cytoplasm is less efficient to induce apoptosis due to its short life span (Alivisatos, 2004; Gao, 2005; Michalet *et al.*, 2005; Smith *et al.*, 2008; Konkar, 2005; Jin *et al.*, 2011)

Allatostatins are pleiotropic insect neuropeptide that *de novo* regulates JH synthesis by corpus allatum endocrine gland and also found to modulate gut motility, muscle contraction and vitellogenesis in insects species (Barbara Stay *et al.*, 1995). This allatostatin neuropeptide biomolecules are found to exist in multiple forms even within the same insect species. Recent studies brought out three different allatostatin isoform families (A-, B- and C-type allatostatins) that inhibited JH biosynthesis have

been identified. The C-type allatostatin also found to inhibit spontaneous peristaltic contractions of insectan foregut. The ALST receptors were found to be expressed in adult *P. americana* in widely different tissues like head, retro cerebral glands, fat body, ovary, male accessory glands, gut, leg muscle, malpighian tubule and nerve cord. This diverse expression pattern clearly denotes its pleiotropic functions of allatostatins extending its role as a paracrine effector (Gade *et al.*, 2008). Two different receptor families (AlstR/DAR-1 and DAR-2) with sequence similarity to mammalian Galanin receptors have been shown to form specific interactions with neuropeptides that resemble cockroach allatostatins (ASTs), with a characteristic Tyr/Phe-Xaa-Phe-Gly-Leu-NH2 carboxyl-terminus noticed.

Allatostatin may be considered to be a carrier peptide to transport guest molecules and materials to the cytoplasm and nucleus of living cells without adversely affecting the physiology of the cell. The coating or conjugation of nanoparticles with allatostatin is a promising strategy for achieving high transfection efficiency. The labeling of microtubules and the nucleus of the cells by QD–allatostatin conjugates would be promising cell labels. Nucleus-targeted photodynamic therapy of cancer in which the generation of reactive oxygen species inside the nucleus induces apoptosis more efficiency than cell membrane- and cytoplasm-specific photosensitization.

Allatostatin is a promising candidate for high efficiency cell transfect ion and nucleus specific cell labeling. Considering the sequence similarity between allatostatin receptors and mammalian somatostatin/galanin cells Biju *et al.*, (2007) demonstrated that in the cancer cell lines significant down regulation of cell proliferation with lower dosages of insect allatostatin neuropeptide. All these implicate the significance of QD-conjugated nanobiomolecules as a valuable bioresources in visualizing the structural dynamics of biological systems *in vivo* as well as *in vitro*.

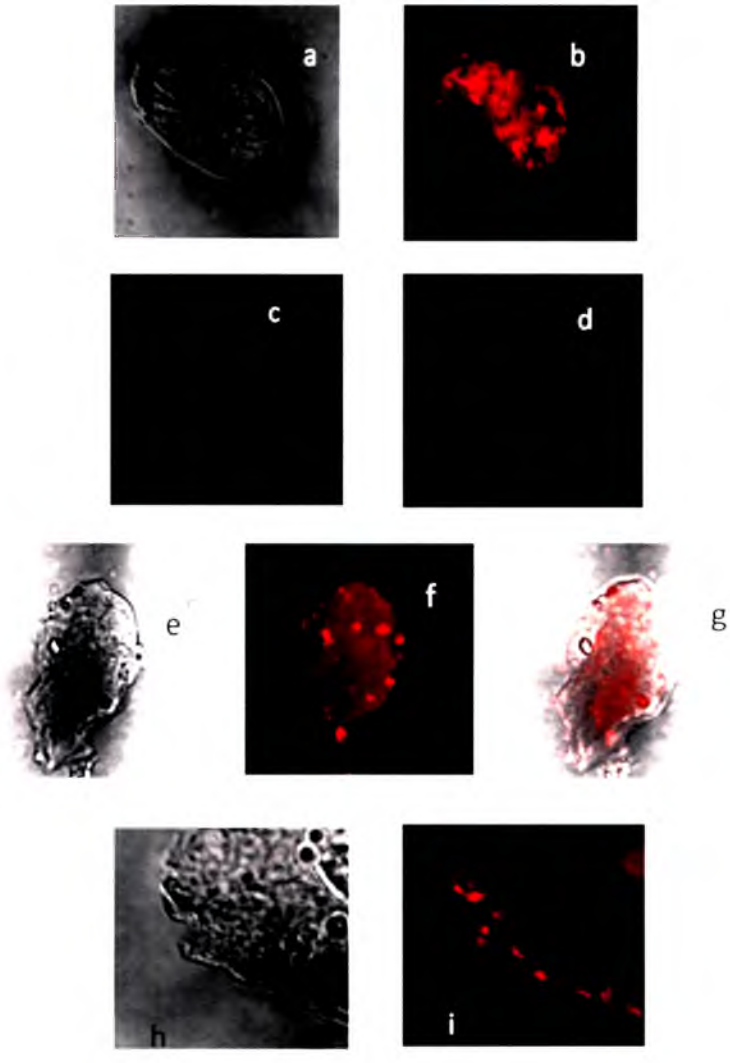
In this context the present study using an allatostatin biomolecule analogue (ALST I) Ala-Pro-Ser-Gly-Ala-Gln-Arg-Leu-Tyr-Gly-Phe-Gly-Leu-NH_2, conjugated to streptavidin-coated CdSe–ZnS QDs to assess its suitability as a viable nanobiomolecular biomarker system. For *in vitro* immunolabelling, Anopheles Stephens mosquito brain dissected out under refrigerated MEM were used. All the experimental mosquitoes used for dissecting out healthy and active whole brain tissue came from the stock colony we maintained under controlled conditions in our laboratory (Temp: 37°C ; R.H. 95 L:D cycle 12:12). All dissections carried out under a stereomicroscope dissection Microscope (Leica). Allatostatin biotinylationv was done by mixing 0.75 mM Alst solution with 3 mM Biotin-N-Hydroxyl succinimide ester solution and kept at room temperature for 1 h. Sephadex G 25 column was equilibrated with Phosphate buffer saline (PBS)n and the reaction mixture containing NHS ester and biotinylated Alst was added to the column. QD conjugates were prepared by simple biotin-streptavidin linkage. A streptavidin-biotin immobilization scheme was selected on the basis that streptavidin-biotin bonds possess high affinity and retain structural stability over a potentially wide pH and retain structural stability over a potentially wide pH ranges. The peptides were biotinylated using biotin-NHS ester and subsequently conjugated to streptavidin functionalized QD at a 1.5 molar ratio.

Brains were incubated with 1 nm solutions of QD peptide conjugates was investigated using Confocal Laser Scanning Microscope. After the reaction mixture is completely absorbed into the column, the eluting solution was discarded. Biotinylated Alst at a concentration of 0.56 mM was eluted from the column using PBS. Alst labeling with quantum dots (QD) was accomplished by mixing 5 μl of 5.6 μM biotinylated Alst, 261 μl deionized water and 14 μl QD together. The total volume of 280 μl reaction mixture contained 100 nM QD and biotinylated allatostatin each. The reaction mixture was kept at 1h at room temperature and under minimum light. Labeling of the Ring Gland (RG) with the conjugant mixture was scone by washing the tissue in PBS followed by incubation in scrum free medium containing QD–Allatostatin Peptide (QD-ALST-Pep) at a concentration of 1 nM for one hour. The immono-labeled RG were visualized under a Confocal Laser Scanning Miscrocope microscope (Leica TCS SP8) excited with 400~520 band pass filter. The fluorescence signal can be viewed using along pass filter (>550 nm) for orange fluorescence.

QD sample (a kind gift from Dr. Biju Vasudevan, AIST, Kagawa, Japan) conjugates were prepared by simple biotin–streptavidin linkage. A streptavidin–biotin immobilization scheme was selected on the basis that streptavidin-biotin bonds possess high affinity retaining structural stability over a potentially wide pH range. The peptides were biotinylated using biotin-NHS ester and subsequently conjugated to streptavidin and QD functionalized at a 1.5 Molar ratio. Dissected out live and active Anopheles Ring Glands (RG) were incubated with 1 nM solutions of QD–peptide conjugate and the image analysis performed using a confocal laser scanning microscope (LEICA CLSM TCS SP2), a Central facility at RGCB, Trivandrum.

Detected the presence of Qdots–AST1 conjugates on the cell membrane, and with time under incubation, the conjugates were found to be endocytosised and imbibed into the cytoplasm and also right inside the nucleus (Plate I (a,b)) while not noticed in the control samples (Plate I (c, d)). As is evident from Plate I (e, f, g, h, i), phase-flurorescence and image-overlay of treated RG brain secretary neurons in contrast to corresponding control samples (Plate I (c, d)) showed end nuclear localization inside the cephalic secretary neurons.

Biological applications of QD employ surface immobilized antibodies and at certain instances peptides are found to passively bind and recognize specific sub-cellular target loci (Simon and Jaiswal, 2004). Such endocytosis and transduction mechanisms for easy intracellular delivery of peptide biomolecules are proposed by Chen *et al.* (2008). Biomolecules like peptides, proteins are often encapsulated into clathrin-coated pits and subsequently taken up in the form of Clathrin coated vesicles (Birbul *et al.*, 1999; Woodhead *et al.*, 1989; Anas *et al.*, 2009). To account for what mechanism is important in the intracellular delivery of QD by allatostatin neuropeptide, the peptide conjugated with CdSe–ZnS QD incubated with the Ring Glands of *Anopheles* mosquito. Lidke *et al.* (2004) have shown that QD conjugated with epithelial growth factor (EGF) showed co-localization with anti active Trk (tyrosine kinase) receptors providing positive indication for QD mediated receptor activation. Conjugated QD-beta subunit of Neurite Growth Factor βNGF, which is smaller than an antibody or a



PLATES I . Fluorescence confocal images of *A. stephensi* whole mount Ring Gland preparations incubated with QD-biotinylated Alst conjugates.
a and **b**: QD–Alst conjugant labeled; **c** and **d**: QD–streptavidin, conjugate free control; **e**,**f** and **g**: Phase-uorescence and overlay images of treated QD–Alst conjugate; **h** and **i**: Ring Gland cephalic secretary neuron specically inside the nucleus showing QD–Alst conjugate entry. Scale bars are 25 m and the images were corrected equally for brightness and contrast.

QD, retained bioactivity and effectively activated TrKA receptors evoking downstream cellular cascades to promote neuronal differentiation (Tania *et al.*, 2005). Alst-I from *Drosophila melanogaster* transfects living NIH 3T3 and A431 human epidermoid carcinoma cells that transports QD right inside the cytoplasm. Live mammalian cells

with QD–Alst conjugates, is found to transport Alst nanobiomolecule inside the cell nucleus and also even enhanced the cell proliferation rate (Biju *et al.*, 2007). Hence QD is indicated to have much potential applications in fluorescencence imaging and hence in biomedicine. Prasad *et al.* (2012) describe the application of microarray gene expression analysis for studying the differential expression pattern of genes using QD. Likewise Gac *et al.* (2006) detected apoptotic cells by conjugating QDs with biotinylated Annexin V, enabling the functionalized QDs to bind to phosphatidyl serine moieties present on the apoptotic cell membrane system but not on healthy or necrotic cells. Wolcott *et al.* (2006) demonstrated silica-coated CdTe QD with functionalized groups for preventing toxic Cd2+ leaking out from the core. Cell-penetrating QDs coated with polyethylene glycol (PEG) grafted polyethyl amine (PEI), capable of penetrating cell membranes and disrupting endosomal organelles in cells (Duan and Nie, 2007). Gold-doped ZnO QDs nanoprobes are used for quick cell detection with very low toxicity (Liu *et al.*, 2011). Allen *et al.* (2010) and Wilson (2010) both propose CdSe/ZnS with PEG coating and CdSe/ZnS-peptide conjugation for cytosol localization and nucleus targeting. Slica-coated Fe3O4 and TGA-capped CdTe QDs nanocomposite was successfully employed for He La cell labelling and imaging, as well as in magnetic separation (Sun *et al.*, 2010). In the light of all the above literature and our present finding venture us to propose as the QD-labeled Alst neuropeptide nanobiomolecule and streptavidin and Immunoglobulin G (IgG) conjugants to target sub cellular nanobiomolecular machinery inside living cells. The present finding further implicate the general capability of ligand–QD conjugates to functionally activate cell membrane receptor biomolecules. Visual tracking and evoking further development probes of cell-specific capability have wide-ranging applications for the progress of molecular tools and therapeutics targeted at indulgent and regulating cell functions.

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